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1st ed in England

THOMAS F. DOUG

IMMUNITY

IN

INFECTIVE DISEASES

CAMBRIDGE UNIVERSITY PRESS WAREHOUSE,
C. F. CLAY, MANAGER.
London: FETTER LANE, E.C.
Glasgow: 50, WELLINGTON STREET.

Leipzig: F. A. BROCKHAUS.
New York: THE MACMILLAN COMPANY.
Bombay and Calcutta: MACMILLAN AND CO., LTD.

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IMMUNITY

IN

INFECTIVE DISEASES

BY

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With 45 figures in the text

CAMBRIDGE
AT THE UNIVERSITY PRESS
1905

Cambridge
PRINTED BY JOHN CLAY, M.A.
AT THE UNIVERSITY PRESS.

PREFACE TO THE ENGLISH EDITION.

IN preparing for the English-reading student this version of M. Metchnikoff's latest work, wherein he "sums up the labours of twenty-five years," it has been my aim to give a faithful rendering of the ideas and argument of the original, even at the risk of an occasional crude expression, rather than to attempt to reproduce the brilliancy of the original by any wide verbal departure from the text.

The Table of Contents forms an admirable analytical summary of the main subject-matters treated, but an alphabetical Index has been added to the present edition, and, though not at all exhaustive, this may serve as a key to the many authors cited and to the maze of detail discussed in the work.

The marginal reference to the pages of the original work will, I hope, commend itself to those readers who may wish to refer to the *ipsissima verba* of the author. It is, I believe, a novelty in scientific works, though familiar in works in other departments of literature.

I am under deep obligations to Professor Woodhead (who has read the whole of the proofs) and to Mr A. E. Shipley, and Mr G. H. F. Nuttall (who have read portions) for much valuable criticism and advice.

THE TRANSLATOR.

August, 1905.

ERRATA.

Page 93, line 2, *after* itself *insert* alone; line 3, *delete* only.
Page 98, line 3 from bottom, *for* actively *read* vigorously.
Page 386, line 16, *for* tortoise *read* turtle.
Page 461, line 21, *after* circumstances *insert reference figure* 1.
Page 537, line 11, *for* arise *read* arises.

TO MESSIEURS E. DUCLAUX AND E. ROUX.

My dear Friends,

Permit me to dedicate to you this work, which sums up the labours of twenty-five years; a very great part of it has been carried out by your side, you who have done so much to lighten my task.

When, nearly fourteen years ago, you allowed me to share your work alongside the venerated Master who founded the House where we have laboured together, you were anything but partisans of my theories; they seemed to you too vitalistic, and not sufficiently physico-chemical. In course of time you became convinced that my ideas were not without foundation, and since then you have given me warm encouragement to pursue my researches in the field that I had marked out for myself.

Working by your side and drawing largely from your vast and varied stores of knowledge, I felt myself safe from those divagations into which a zoologist, who had wandered into the domain of biological chemistry and of medical science, is likely to stray. I thank you with all my heart, and I beg you to accept the homage of this work as a testimony of my deepest gratitude and of my warmest friendship.

ÉLIE METCHNIKOFF.

Institut Pasteur,
3 *October*, 1901.

PREFACE.

WHEN, ten years ago, I was preparing my *Lessons on the Comparative Pathology of Inflammation* for the press, I hoped that the other sections of the phagocytic theory—Immunity, Atrophies, and Healing—would soon follow this first work. This hope has not been realised, and it has needed prolonged work ere I could publish the volume I have just completed.

During this long period I sent out several *ballons d'essai* under the form of summaries of the question of Immunity, published in the *Semaine médicale* (1892), the *Ergebnisse* of Lubarsch and Ostertag (1886), and the *Handbuch der Hygiene* by Weyl (1897). I there attempted, as far as possible, to give a general picture of the phenomena of Immunity in the infective diseases, and it was my desire to excite criticism and opposition, in order to determine the fate of the theory of phagocytes in its application to the problem of Immunity.

The most recent attempt in this direction was made at the International Congress at Paris, in the past year (1900), when I presented my report on Immunity before an audience which included, amongst others, my principal opponents. It was the result of this Congress which at length decided me to bring together my views on Immunity in a volume which I now present to the reader.

Convinced that many of the objections raised against the phagocytic theory of Immunity proceeded solely from an insufficient knowledge of the theory, I thought that a work condensed into one volume might render some service to those who are interested in the problem of Immunity. I do not know whether I shall convert my opponents, but I am convinced that a perusal of this book will clear away certain misunderstandings. A very competent observer recently confessed in one of his publications that for many years he had been unaware of the experiments of M. J. Bordet and myself on Immunity against the cholera vibrio, experiments which he now

regards as of fundamental importance for the comprehension of the whole question of Immunity. I hope that after the appearance of this treatise such oversights will not be so likely to occur.

Should I not succeed in convincing my opponents of the justice of the cause which I defend, I shall at least have informed my critics and shall have given them an opportunity of discussing it with a thorough knowledge of the material on which it is based. This result alone would justify me in having undertaken this work.

At first I intended to add to my explanation of Immunity a theory of the phenomena of healing in infective diseases, but I soon had to renounce this project, for its execution would have increased too greatly the bulk of the book which, without it, has already assumed considerable proportions. It seemed to me preferable to set forth the present state of the question without paying too much attention to the historical sequence of the discoveries, and to reserve for a special chapter, at the end of the work, a sketch of the history of our knowledge on Immunity.

Before I ask the reader to glance through this work, I should mention that I have been heartily seconded in its preparation by many of my friends and collaborators. I offer my most sincere thanks to MM. Roux, Nocard, Massart, and J. Bordet, who kindly undertook to read my manuscript throughout, or such parts of it as related to their special subjects. For example, M. Nocard rendered me a very great service by correcting the paragraphs of Chapter XV, which treat of the vaccinations against epizootic diseases, and M. Massart, by giving me his advice on the subject of immunity in plants.

I owe very special thanks to M. Mesnil, who has been good enough to give me very effective help in the dry task of correcting the manuscript and proofs.

I beg MM. E. Rémy and L. Barnéoud to accept my thanks for the care they have bestowed on the execution of the illustrations in this work.

ÉLIE METCHNIKOFF.

PARIS, INSTITUT PASTEUR,
3 *October*, 1901.

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THE problem of immunity in relation to infective diseases is one that not merely concerns general pathology but has a very important bearing on all branches of practical medicine, such as hygiene, surgery and the veterinary art. The prevention of disease by the production of an acquired immunity is daily assuming greater importance. With the object of arresting the multiplication and dissemination of morbific germs, we are seeking, by artificial means, to render individuals, who may come in contact with them, refractory to their pathogenic action. Patients who have just undergone a surgical operation and women in child-bed are frequently in danger of acquiring a post-operation disease or a puerperal affection; we are, therefore, striving to protect them by conferring upon them an artificial immunity.

The immunisation of animals useful to man is likewise a question of such great importance to agriculture and to industry as to have now become the object of legislation.

This question of immunity is, however, apart from its practical aspect, intimately connected with problems of pure theory. There can be no question that the marked pessimism developed during the century just closed was in a large measure prompted by the dread of disease and premature death, scourges against which humanity is as yet powerless. It is recognised that Byron and Leopardi, the great poets of pessimism, both suffered from congenital anomaly and from incurable disease and that these maladies cast a gloom over their poetry. Schopenhauer, the founder of the

[2] pessimistic school in modern philosophy, was noted for his exaggerated fear of disease.

During the greater part of the nineteenth century our knowledge as to immunity has been limited to certain practical methods, often efficacious it is true, but purely empirical, such as those employed in immunising man against small-pox and certain domestic animals against sheep-pox or pleuro-pneumonia.

So long as the nature of the viruses was unknown no really scientific study of their action or of immunity from them could be made. The revelation of the organised nature of the infective viruses opened up the way for these researches. This discovery, the outcome of the demonstration by Pasteur of the organised nature of the ferments, has enabled us to establish the part played by living agents in a great number of infective diseases, and, linked with the names of Davaine, Obermeyer, and above all with that of Robert Koch, it has very greatly advanced the study of susceptibility and of natural immunity in certain infections.

A considerable forward step was made with the discovery, by Pasteur and his collaborators Chamberland and Roux, that it was possible, in certain infective diseases, to confer immunity by means of micro-organisms which had had their virulence attenuated. Thanks to this discovery, science was now in a position to take up the thorough study of acquired immunity. The field of research was still further enlarged by the demonstration of the immunising power of the culture-products of pathogenic micro-organisms and above all by the discovery that the blood of immunised animals is capable of conferring immunity upon susceptible animals.

Before taking up in detail the problem of immunity as it is revealed to us as a consequence of these discoveries, it is essential to cast a glance at infective and allied diseases as a whole and to indicate in what light we look upon them in view of the present state of our knowledge.

It has been definitely established that many infective diseases of man and animals are due to the invasion of small parasitic organisms, sometimes of animal nature (as in itch, trichinosis, malaria, Texas fever, nagana, or surra and the allied condition "dourine" in horses), sometimes belonging to the vegetable kingdom like the Moulds (aspergillosis), the Hyphomycetes (actinomycosis, Madura foot
[3] disease, bovine farcy) and the Yeasts (disease of the *Daphniae*, some pseudomyxomas and septicaemias, pseudolupus). But by far the

greater number of infective diseases are due to the development in the organism of plants of the simplest structure, Bacteria. These Bacteria produce the gravest and most destructive infections, such as tuberculosis, bubonic plague, diphtheria, cholera, anthrax, the pneumonias, suppuration, erysipelas, tetanus, glanders, leprosy, &c. Among these bacteria some are too small to be resolved individually under the highest magnifying powers and can only be made out *en masse*. Such is the micro-organism of the contagious pleuro-pneumonia of cattle. To this minuteness of certain pathogenic Bacteria is very probably due the fact that in a considerable number of infections, amongst which are scarlatina, measles, rabies, syphilis, aphthous fever and small-pox, it has been impossible, up to the present, to recognise any specific micro-organisms.

It is probable that we shall succeed in discovering parasites, not only in the diseases I have just cited, which present the characters of infective and virulent diseases, but also in diseases of entirely different types. In spite of the failure of various attempts to demonstrate the parasite of malignant tumours, it may be hoped that, with improvement in scientific methods, such a parasite will be unequivocally demonstrated. In many other conditions which are at present considered as not dependent on micro-organisms, an intimate connection with such organisms will probably be established. Such are the atrophic diseases and certain diseases of nutrition in which the parasites, without playing a direct or immediate *rôle*, act by means of their products, or by the changes which they set up in the affected organism. To give an idea of this possibility it will be useful to cast a glance at the various modes of action of the numerous etiological agents in infective diseases. The parasites which produce them have, as a common feature, their small dimensions; they can only be recognised with precision by the employment of high powers of the microscope. They are likewise distinguished by a great variability, which is not astonishing, since among infective agents are found on the one hand animals of high structure (such as the Acari of itch) and on the other plants of the simplest character such as the Gonococci or the various Cocco-bacilli.

The Acari are capable of perforating the epidermis by the mech- [4]
anical action of their feet and mouth-parts. They excavate channels in the skin and thus provoke the irritation so characteristic of itch. The larvae of the Trichinae in like manner produce marked lesions by the mere mechanical act of penetration and migration in the striped

fibre of muscular tissue. In human trichinosis, however, the disease picture is more complicated than in itch and leads us to assume that there is some additional action of the excreta of the larvae in the production both of the febrile state and of certain general phenomena. In the nagana disease (transmitted by the Tsetse fly) there is equal reason to admit the preponderating *rôle* of the mechanical action of the flagellated parasite (*Trypanosoma*) which obstructs the vessels of the nervous centres.

In the diseases which are set up by Fungi, such as ringworm and aspergillosis, the purely mechanical element still appears to play the more important part. Even certain of the bacterial infections manifest this same character. Thus, there is no doubt that in chronic tuberculosis in the guinea-pig, Koch's bacillus brings about a substitution of tuberculous elements for the normal tissues, and this to such a degree that, at the termination of the disease, there may remain merely traces of the liver and of the lungs, and the animal dies for want of these organs, whose normal action is no longer possible. In the tuberculous guinea-pig the phenomenon of intoxication by the bacillary poisons plays but a secondary *rôle*; yet there are examples of tuberculosis (as in acute miliary tuberculosis in man or experimental tuberculosis in cattle, obtained by Nocard's method of inoculation into the milk ducts), where the poisoning assumes much greater importance.

Among the bacterial diseases of man, leprosy may be cited as one in which the intoxication is relegated to a subsidiary position, yielding place to the mechanical substitution of the specific granuloma for the normal tissues. It is only in the acute leprous exacerbations that we perceive any signs of intoxication by the products of the leprosy bacillus.

All the instances cited, however, constitute but a feeble minority which is completely thrown into the shade in the presence of very numerous infections in which the toxic element dominates the situation. Even in carbuncular diseases an exact analysis of their morbid phenomena has compelled us to recognise the marked influence of the
[5] poison produced by the bacterium. The majority of the micro-organisms act as poisoners which introduce themselves into the organism where they can secrete toxins capable of provoking general disorders of very diverse natures. Indeed in infective diseases a whole gamut of very remarkable variations is produced. Thus many of the micro-organisms capable of setting up septicaemias must multiply

abundantly in the organism and be distributed in the blood, before they can produce a general morbid condition. The spirillum of human recurrent fever is an example of this. It multiplies for some days and produces several generations without provoking the least malaise; then, however, their appearance in the blood suddenly produces intense fever and constitutional phenomena of the most pronounced character.

On the other hand there are certain bacteria which are distinguished by a very much feebler reproductive power, but a more marked toxic activity. Incapable of spreading through the organism, these bacteria remain localised at the point of entrance, where they secrete their poisons and thus frequently set up a fatal intoxication. Some of these bacteria, such as the bacilli of tetanus and of diphtheria, penetrate more or less deeply into the living tissues of the affected animal. Others can manifest their toxic action so to speak at a distance or by simple contact with the living elements. Into this category comes the organism of Asiatic cholera. Koch's vibrio, once established in the intestine, there secretes its poison; this, absorbed by the apparently intact intestinal mucous membrane, sets up a fell disease, purely toxic in character. It is probable that in the case of those intestinal diseases whose etiology is still unknown, such as infantile choleras, the poisoning by the products of micro-organisms constitutes the essential phenomenon. The micro-organisms do not make their way into the blood or tissues; they remain in the contents of the intestine and thence set up their deadly intoxication.

Instances do exist in which the pathogenic micro-organism disappears from the body, leaving there a toxin which, alone, is responsible for death. Thus in the spirillar septicaemia of geese, the birds die at a stage when not a single living spirillum can be found in the body. The poisoners have been destroyed before the toxin produced by them had completed its work. In other instances, e.g. typhoid fever of the horse, the specific micro-organism likewise disappears before the death of the animal; but at the period when the poison of this bacterium finishes its fatal work, there is a secondary invasion of [6] other micro-organisms which have nothing to do with the typhoid fever proper of the horse.

This great variability in the action of the different pathogenic agents is still further increased through the differing relations between the parasites and the affected organism. Certain micro-organisms are capable of producing a typical disease, whatever may be the

mode and seat of invasion of the organism. But these are comparatively few in number. The bacillus of tuberculosis belongs to this minority. Whether it enters subcutaneously, by the eye or by the respiratory, digestive or genito-urinary passages, it invariably produces tubercular lesions more or less grave and more or less capable of generalisation. On the other hand, a very large number of micro-organisms only exert their pathogenic action when they invade the organism at definite points. The anthrax bacillus, when introduced through the slightest lesion of the skin or of the mucous membranes, produces in man, and in a large number of mammals, a very grave and usually fatal disease; when absorbed in the vegetative state with food, it is almost always innocuous. With the cholera vibrio we have an exactly opposite condition of affairs. When inoculated, even in large numbers, below the skin in the human subject, it rapidly disappears, producing merely insignificant disturbances; but when the same vibrio is introduced into the digestive canal it develops and produces Asiatic cholera, a disease so often terminating in death.

All these variations and peculiarities associated with the nature of infective agents are of great importance from the point of view of immunity.

Do diseases come from without or do their causes arise within the organism? is a pressing question, long discussed by pathologists. Those who have discovered most of the pathogenic micro-organisms have ranged themselves on the side of the former hypothesis. For the majority of them the essential etiological factor in the causation of infective diseases consists in the invasion of the patient by the pathogenic micro-organism from the outer world. This theory is in perfect harmony with many of the admitted facts of epidemiology, according to which the viruses of the most deadly epidemic diseases, such as Asiatic cholera, yellow fever, and bubonic plague, must be imported into a country previously free from the disease before an epidemic can be developed. In anthrax and trichinosis it is recognised
[7] that the parasites must come from without. Hence, in the study of pathogenic micro-organisms one always follows the rule that it is essential to find the specific micro-organism in all cases of the disease in question and to prove its absence in healthy individuals or in those who are affected with other diseases. Thus, Koch[1], in his classical researches on Asiatic cholera, insisted on the fact that the cholera vibrio was always found in cases of this disease but never in healthy

[1] *Deutsche med. Wchnschr.*, Leipzig, 1884, SS. 499, 519.

persons. Almost simultaneously Loeffler[1], in the course of his work on the etiology of diphtheria, demonstrated the presence of a specific bacillus not only in a large number of cases of this disease but also in the throat of a healthy child; and this fact at first prevented him from accepting this bacillus as the real cause of diphtheria.

This view accepted by two such eminent bacteriologists cannot however be maintained. It is impossible to assume that each time that a pathogenic micro-organism makes its way into a susceptible species its presence must inevitably be followed by the production of the specific disease. Although the discovery by Loeffler of the diphtheria bacillus in the throat of healthy individuals has repeatedly been confirmed, it is impossible to doubt the etiological *rôle* of this organism in diphtheria. Moreover, it has been established that Koch's vibrio, although undoubtedly the etiological factor in the production of Asiatic cholera, has nevertheless been recognised in the digestive canal of perfectly healthy persons.

As soon as he is born, man becomes the habitat of a very rich microbial flora. The skin, the mucous membranes, and the gastro-intestinal contents become stocked with such a flora, but a very small number of these micro-organisms have up to the present been recognised or described. The buccal cavity, the stomach, the intestines and the genital organs offer a feeding ground for Bacteria and inferior Fungi of various kinds. For long it was thought that in healthy individuals all these micro-organisms were inoffensive and sometimes even useful. It was supposed that when an infective malady was set up a specific pathogenic micro-organism was added to this benign flora. Exact bacteriological researches have, however, clearly demonstrated that as a matter of fact the varied vegetation in healthy persons often includes representatives of noxious species of [8]
bacteria. Besides the diphtheria bacillus and the cholera vibrio, which have repeatedly been found in a virulent form in perfectly healthy individuals, it has been demonstrated that certain pathogenic micro-organisms, e.g. the *Pneumococcus*, staphylococci, streptococci and the *Bacillus coli*, are always, or almost constantly, found among the microbial flora of healthy persons.

This observation has necessarily led to the conclusion that in addition to the micro-organism there exists a secondary cause of infective diseases—a predisposition, or absence of immunity. An individual in whom one of the above-mentioned pathogenic species is

[1] *Mitth. aus d. K. Gesundheitsamte*, Berlin, 1884, Bd. II S. 421.

present, manifests a permanent or transitory refractory state as regards this specific organism. As soon however as the cause of this immunity ceases to act, the micro-organism gets the upper hand and sets up the specific disease. It is thus in diabetic persons that boils make their appearance as the result of the development of *Staphylococcus pyogenes*, a micro-organism that is almost always found in abundance on the skin and mucous membranes of the human subject. The diabetes is, in these cases, the cause of the suspension of the immunity which exists in the healthy individual.

People who carry the *Pneumococcus* on their mucous membranes may remain for long without being attacked by fibrinous pneumonia or any of the other maladies due to this micro-organism. But often, in consequence of some special circumstance, a cold for example, the refractory state gives way to a more or less marked susceptibility.

It is unnecessary to multiply the number of such examples; they demonstrate in the clearest fashion that, in addition to the causes of disease which come from the outer world and which are represented by the micro-organisms, there are yet other causes which lie within the organism itself. When these internal factors are powerless to prevent the development of the morbific germs, a disease is set up; when, on the other hand, they resist the invasion of the micro-organisms properly, the organism is in a refractory condition and exhibits immunity.

Diseases in general and infective diseases in particular were developed on the earth at a very remote epoch. Far from being peculiar to man, animals and the higher plants, they attack inferior forms and are widely distributed among unicellular organisms, In-
[9] fusoria and Algae. Diseases undoubtedly play an important *rôle* in the history of life on our planet, and it is very probable that they have contributed in a marked degree to the extinction of certain species. When we observe the ravages produced by parasitic Fungi among the young fish which we are trying to rear, or the destruction of crayfish in certain countries in consequence of the rapid increase of epizootic germs, we are involuntarily led to the conclusion that pathogenic micro-organisms must have brought about the disappearance of certain animal and vegetable species.

Darwin[1], in the chapter on the extinction of species in his book *On the Origin of Species*, states upon the authority of several observers that insects so annoy elephants that these large mammals

[1] "On the Origin of Species," 6th ed., London, 1872, Chapter XI, p. 277.

become incapable of reproducing themselves in sufficient numbers. Now it is proved that many Insects inoculate pathogenic micro-organisms and thus transmit destructive diseases. A most formidable epizootic disease, provoked by a flagellated Infusorium, the *Trypanosoma brucei*, is inoculated into large mammals in South Africa by a fly, the Tsetse fly; in certain districts this disease is so widespread and so destructive that the rearing of domestic animals becomes impossible.

Parasites strike then with great intensity, bringing about the destruction of numerous human beings, animals and plants. Nevertheless, in spite of the disappearance of a large number of species, the world continues well populated. This fact proves that, by the special means at the disposal of the organism, without any aid of the medical art or special human intervention, many living species have held their own throughout the ages. Everybody has seen how dogs lick their wounds, moistening them with a saliva full of micro-organisms. These wounds heal well and quickly without dressings or antiseptics.

In all these examples the resistance of the organism depends on immunity, a condition very general in nature. This immunity against infective diseases is very complex and its thorough study could only be undertaken after we had acquired an extended knowledge of these diseases, and after adequate methods of research had been devised.

By immunity against infective diseases we understand the re- [10]
sistance of the organism against the micro-organisms which cause these diseases. We have here to do with an organic property of living beings and not with the immunity which belongs to certain countries or localities. For this reason information on the causes of the immunity in Europe and in mountainous regions from yellow fever will not be found in this book, nor why the majority of Europeans do not take recurrent fever. The inhabitants of our continent do not possess organic immunity against either the virus of yellow fever or Obermeyer's spirillum of recurrent fever. Indeed they are very susceptible to these diseases. It is solely the conditions of life, in the majority of European countries, that prevent the invasion by the specific germs and the creation of epidemic foci. The same point of view ought also to be applied to animals. Our small laboratory rodents, mice and guinea-pigs, are much more susceptible to anthrax, whether inoculated beneath the skin or

in any other part of the body, than are the large domestic mammals such as the ox and the horse. And yet these latter are very liable to epizootic anthrax, whilst the rodents mentioned are seldom, if ever, attacked by spontaneous anthrax. This apparent immunity in no way depends on the existence of a true immunity of the organism, but solely on the conditions under which mice and guinea-pigs live.

We shall therefore in this volume treat only of the phenomena of *organic* immunity in living beings, and the problem, even restricted within these limits, still appears sufficiently complex. With the object of rendering its study as easy as possible, it will be useful to commence by giving an account of the phenomena of immunity in the lowest organisms.

Immunity against infective diseases should be understood as the group of phenomena in virtue of which an organism is able to resist the attack of the micro-organisms that produce these diseases. It is impossible, at present, to give a more precise definition, and useless to insist upon it. Some have thought it necessary to distinguish between immunity properly so called, that is to say a permanent refractory state, and "resistance," or a very transient property of opposing the invasion of certain infective micro-organisms. It is not possible to maintain this distinction, for in reality the limits between these two groups of phenomena are far from being constant.

[11] Immunity may be inborn or acquired. The former is always natural, that is to say, independent of the direct intervention of human art. Acquired immunity is also often natural, from the fact that it is established as the result of the spontaneous cure of an infective disease. But in a great number of cases acquired immunity may be the result of direct human intervention as in the practice of vaccination.

For a long time all the phenomena of immunity against infective diseases were collected into a single group. Later, it was recognised, as the result of the demonstrations summarised at the beginning of this chapter, that it is necessary to distinguish sharply between immunity against the pathogenic micro-organisms themselves and that against microbial poisons. Hence the idea of antimicrobial and antitoxic immunities. In the course of this work this essential distinction must always be borne carefully in mind.

CHAPTER I

IMMUNITY IN UNICELLULAR ORGANISMS

Infective diseases of unicellular organisms.—Intracellular digestion in the Protozoa.— [13]
Amoebo-diastase.—Part played by digestion in the defence of the Protozoa against infective parasites.—Defences of the *Paramaecia* against micro-organisms. —Part played by irritability in defence in the lower organisms.
Immunity of unicellular organisms to toxins.—Acclimatisation of Bacteria to toxic substances.—Protective secretion of membranes by Bacteria.
Adaptation of the Protozoa to saline solutions—of yeasts to poisons—of yeasts to milk-sugar.
Irritability of unicellular organisms and Weber-Fechner's psycho-physical law.

THE immunity of unicellular organisms against infective diseases and against toxic agents is as yet very imperfectly understood. Nevertheless, it will be very useful for us to begin our study of the problem of immunity on these lower organisms, because of their greater general simplicity. It may be affirmed that if the line of comparative pathology had been followed in our study of the etiology of diseases of man and the higher animals, the parasitic nature of these infections would have been established considerably earlier than was the case. Thus, at a period when medical men and veterinary surgeons were content to record the presence of Bacteria in the blood of their patients, without attributing to them the slightest etiological *rôle*, botanists and zoologists had already proved most definitely that many plants and lower animals were subject to epidemic diseases undoubtedly set up by the parasitism of various exceedingly simple organisms. In the same year, 1855, that Pollender[1] published his first observations on the bacterium found in the blood of animals affected by anthrax though he could not trace the slightest relation between the presence of this organism and the etiology of the disease, the

[1] *Vrtljschr. f. gerichtl. Med.*, Berlin, 1855, S. 102.

celebrated botanist Alexander Braun[1] issued his work on the genus
[14] *Chytridium*, in which he demonstrated the fact that certain plants and flagellated Infusoria suffer from the invasion of a small mobile parasite which, attaching itself to their body-wall, absorbs the contents and so destroys its hosts, causing a very great mortality among them. The cycle of development in the *Chytridia*, established by Braun, left no doubt as to the accuracy of his view and even renders it possible for us to interpret more accurately the earlier observations of Stein, on the supposed evolution of certain Infusoria, by showing that the changes observed in these organisms were in reality due to an invasion by *Chytridia*.

Since these observations were made it has been clearly demonstrated that among the unicellular organisms, certain Flagellata and ciliated Infusoria are subject to infective maladies the result of parasitism of the Chytridiaceae, a group of the lower Fungi. Small, mobile, colourless cells attach themselves to the surface of the Protozoa, penetrate into their interior and absorb the greater part of their living content. Sometimes these parasites multiply in a most extraordinary fashion and destroy enormous numbers of the Infusoria. Thus, Nowakowski[2], who has given a very detailed description of *Polyphagus euglenae*, the Chytridium of the common green fresh-water *Euglena*, records the disappearance of the *Euglenae* from his aquaria glasses: the parasites "were reproduced in such great abundance that ultimately they had completely replaced the *Euglenae*."

The Flagellata, subject to infection by *Chytridia*, are found almost exclusively amongst those genera (*Cryptomonas*, *Chlamydomonas*, *Haematococcus*, *Phacus*, *Volvox*, etc.) which are nourished after the fashion of vegetables, that is by the absorption of substances dissolved in the surrounding fluids. It is very remarkable that in the group of ciliated Infusoria this parasitism of the *Chytridia* is observed almost solely in the encysted forms, that is to say, at a stage when the animalcules, surrounded by their envelope, do not take any nourishment. The invasion by the *Chytridia* has been demonstrated in the case of the cysts of the Vorticellina, Oxytrichinina, *Nassula*, etc.[3] These facts indicate that the absence of the digestion of solid aliments, such as occurs in almost all the ciliated Infusoria, con-

[1] "Ueber Chytridium," in *Monatsber. d. Berliner Akad.*, 1855, June, No. 14.

[2] Cohn's "Beiträge zur Biologie der Pflanzen," Breslau, 1876, Bd. II, S. 210.

[3] For the parasites of Infusoria, cf. Bütschli in Bronn's "Klassen und Ordnungen d. Thier-Reichs," Leipzig, 1885—1889, Bd. I, SS. 872, 1823, 1944.

stitutes a condition favourable to infection by the *Chytridia*. Whilst [15] the growth of Volvocina, *Euglenae* and their allies is almost always interfered with by very destructive parasitic epidemics, the ciliated Infusoria, capable of seizing and digesting lower organisms, may be cultivated and flourish for a very long period. Thus Balbiani[1] has watched one of his cultures of *Paramaecium aurelia* multiply and thrive in splendid condition for 14 years in succession. Now these Infusoria readily adapt themselves to ordinary water untreated to render it more hygienic. Such water swarms with all sorts of lower organisms, among which are the *Chytridia* and numerous Bacteria, but the *Paramaecia* and Infusoria in general feed upon these organisms and contribute largely to the purification of the water. Almost the whole body-contents in a ciliated Infusorian is made up of a digestive protoplasm into which the captured Bacteria and other lower organisms are conveyed; the nutrient particles becoming surrounded by transparent vacuoles, in which the ingested organisms are killed and digested. The food contained in the vacuoles circulates in the endoplasm of the Infusoria by means of the streaming movements of this layer. The digestive vacuoles become filled with a fluid having a distinctly acid reaction. Formerly, in order to demonstrate this reaction, Infusoria were allowed to ingest small granules of blue litmus which after a certain time became more or less intensely red; but the use of aniline colours has much simplified the study of digestion in microscopic organisms. By introducing a solution of alizarin sulpho-acid into a liquid containing Infusoria, the yellow staining (characteristic of the acid reaction) of the digestive vacuoles can be readily made out. When the Infusoria ingest small clumps of alkaline substances, stained violet by this reagent, the vacuoles take on a red tint, indicating the acidity of their contents[2]. Another aniline colour, neutral red (Neutralroth), introduced into microscopical technique by Ehrlich[3], enables us to demonstrate the acid reaction in the digestive vacuoles even within a few minutes. Thus, in *Paramaecia* treated with a dilute solution of this reagent, the digestive vacuoles at once assume the deep rose tint, characteristic of an acid reaction. This coloration is observed during the life of the Infusorian, but immediately after death [16]

[1] *Arch. d'anat. microsc.*, Paris, 1898, t. II, p. 528.

[2] Le Dantec, "Recherches sur la digestion intracellulaire," Lille, 1891, p. 53.

[3] Ehrlich u. Lazarus, "Die Anämie," in Nothnagel's "Specielle Pathologie u. Therapie," Wien, 1898, Bd. VIII, I[ter] Theil, S. 85; also "Pathology of the Blood," authorised English translation, Cambridge, 1900, p. 125.

the vacuoles become brownish and then completely lose their colour. This reaction, easily demonstrated, indicates that neutralisation of the acid of the vacuoles by the protoplasm and the surrounding water, both of which are alkaline in reaction, has taken place.

In a medium distinctly acid the Infusoria digest their prey which, in a very great number of cases, consists of Bacteria. These micro-organisms are swallowed and carried into the digestive endoplasm in the living condition; we have evidence of this in the active movements of a certain number of the bacteria; at first they are found isolated in the interior of the vacuoles, but later they collect into more or less compact clumps. These masses of micro-organisms undergoing digestion, when treated with neutral red assume a very deep rose tint, preserving their bacillary form to the end, that is to say up to the extrusion of the effete or waste material. There is, indeed, only very imperfect dissolution not only of the bacilli as a whole but also of their contents. *Paramaecia* placed amongst cholera vibrios swallow them greedily and in great numbers, digesting them as they would any other micro-organism. I have never been able to see any conversion of vibrios into granules going on within the digestive vacuoles.

All the attempts that have been made in my laboratory to extract a digestive fluid from *Paramaecia* have failed entirely. Very large quantities of these Infusoria, obtained by filtration of rich cultures, and macerated by different methods, have proved inactive even in the case of those Bacteria which constitute their normal food.

Intracellular digestion in the Infusoria unquestionably takes place as the result of the action of some diastase; but from the impossibility of observing the action *in vitro* the properties of this diastase, except that it can act in a distinctly acid medium, cannot be determined.

Even less is known concerning the digestion of Rhizopods than concerning that of Infusoria. It has long been recognised that, in the majority of cases, *Amoeba, Actinophrys* and Rhizopods in general, absorb a nourishment composed of lower plants and animals, which are taken into the protoplasmic body by means of the movements of amoeboid processes, pseudopodia or lobopodia.
[17] Once within the Rhizopod the nutritive particles are surrounded by a digestive fluid, in which the presence of acid may be recognised by means of colour reactions. The addition of a drop of Ehrlich's neutral-red to *Amoebae* in the act of digesting Bacteria

at once gives the acid colour reaction (Fig. 1). Rhumbler[1] has described very precisely and with much detail the way in which the *Amoebae* behave when they are incorporating filaments of *Oscillaria* very much longer than their own bodies. He has also described the digestion that these Algae undergo; a process most characteristic in those cases where a portion only of the filament has been taken into the interior of the *Amoeba* and there subjected to the digestive action. Whilst the free part of the *Oscillaria* retains its normal properties and appears of a bluish green colour, the ingested portion progressively changes colour, assuming first a deep green tint, then becoming light yellow, orange yellow, brown and finally reddish brown. Simultaneously the cellulose wall of the Alga begins to soften, and the cells break up into minute fragments which are soon extruded. The food is seldom completely digested and there is always an abundant residual material which is thrown out in the form of solid excreta.

Fig. 1. An *Amoeba* treated with neutral-red, 1 %.

Although it is fully recognised that, in the Rhizopods, digestion goes on in a medium distinctly but feebly acid, and that the intervention of some soluble ferment is essential, our ideas on this subject were very vague until the publication of the researches of Mouton[2], carried out with great care in the Pasteur Institute. In order that he might obtain exact results Mouton made use of cultures of *Amoebae* grown on agar, in association with the *Bacillus coli* which served them as food. The bacilli were ingested in large numbers, became enclosed in vacuoles and were digested by a ferment which Mouton was able to obtain *in vitro*. To that end he collected large numbers of *Amoebae*, and, after centrifugalising them in water, treated the deposit with glycerine. On adding alcohol [18] he obtained a precipitate readily soluble in water.

The fluid thus obtained exerted an undoubted digestive action upon albuminoid substances. It readily liquefied gelatine and even attacked, though feebly, albumen coagulated by heat; flakes of fibrin heated to 58° C. remained unaltered. There was present then, in this fluid derived from *Amoebae*, a proteolytic diastase of feeble activity. On the other hand, this extract contained neither sucrase,

[1] *Arch. f. Entwickelungsmech.*, Leipzig, 1898, Bd. VII.
[2] *Compt. rend. Acad. d. Sci.*, Paris, 1901, t. CXXXIII, p. 244.

capable of inverting cane sugar, nor lipase, capable of digesting fatty matters.

The amoebo-diastase of Mouton must be classified with the trypsins. It is very active in a distinctly alkaline medium and continues the diastatic action even when the medium becomes weakly acid (a feature that corresponds to the reaction observed in *Amoebae* treated with appropriate staining agents). The amoebo-diastase is affected at as low a temperature as 54° C. and at 60° C. is rendered completely inactive.

A question of especial importance is that concerning the action of the amoebo-diastase upon Bacteria. The numerous experiments of Mouton directed to the solution of this point, and made with living *Bacillus coli communis*, gave negative results. If, however, these bacilli were previously killed by heat or by chloroform, they were at once attacked by the soluble amoebo-ferment. Opalescent emulsions of these dead bacilli, incapable of undergoing self-digestion of any kind, became transparent after remaining for some time in contact with the extract of *Amoebae*. The amoebo-diastase, then, undoubtedly digests dead bacilli *in vitro*, whereas in the body of the *Amoebae* the ingested bacteria are attacked whilst still living. As a result of these observations it must be concluded that only a fractional part of the diastase is extracted in the solution prepared by Mouton.

This intracellular digestion in the Protozoa serves not merely for the nutrition of these organisms, but also as a protection against infective parasites. The protoplasm of the Infusoria, with its vacuolar secretions, has a general digestive action on everything that comes within its reach. If the internal structures, such as the nuclei and the pulsatile vacuoles, resist this process, it is undoubtedly because they possess a power of defending themselves against the attack of the digestive secretions. Thus, as brought out in the beautiful
[19] researches of Maupas[1], the macronucleus of the *Paramaecia* is, at a certain stage in the life of the Infusorian, completely digested by the protoplasm just as is any other nutrient substance introduced from outside. It must be admitted that in this case the nucleus has ceased to produce the protective substance which, under ordinary conditions, interferes with its being digested.

A struggle similar to that observed between the nucleus and the digestive content of the Protozoa goes on between these latter

[1] *Arch. de zool. expér.*, Paris, 1889, 2me série, t. VII, p. 446.

organisms and infective microbes. All organisms which, in any way whatever, penetrate into the body of an Infusorian or Rhizopod, are brought into contact with the digestive endoplasm of these Protozoa. If the intruders are killed and partially digested by the digestive secretions, or are expelled as excrementitious matter, the Protozoon remains uninjured and continues its normal and routine existence. Here, then, we have an example of natural immunity, due to intracellular digestion. On the other hand, when the foreign parasitic organism resists this digestive action, it instals itself permanently in the body of the Protozoon, and should it reproduce itself in small numbers merely, excrete no poison and, in general, exercise no injurious influence upon its host, the parasite may readily become a commensal. Thus, it is not rare to find in the contents of Infusoria and Radiolaria small vegetable organisms of the genera *Zoochlorella* or *Zooxanthella* which not only set up no disease but, owing to their assimilation of carbonic acid, may even be useful to their hosts. There are cases, however, where the parasites act in a manner more or less injurious to the Protozoa containing them; in such cases a true and sometimes fatal infection results.

Among the infective diseases of the Protozoa the one that has been most thoroughly studied is that set up by several representatives of a particular genus of micro-organisms discovered by Johannes Müller in 1856 and made the subject of an investigation carried out in my laboratory by Hafkine[1]. I have already discussed these researches in my work on the comparative pathology of inflammation[2] and need here recapitulate only very briefly. *Paramaecia* are sometimes affected by needle-shaped or spirillar parasites which penetrate, sometimes into the macronucleus, sometimes into the micronucleus, reproducing prolifically, giving rise to a marked hypertrophy of the affected organs. The Infusorian, in spite of this invasion, may continue to exist and carry on its reproductive processes; it is, thus, enabled in many cases [20] to recover from the disease. On the other hand the *Paramaecium* into whose body the spores of the parasite are introduced treats them as it would any other ingested foreign body. Not being able to digest them, owing to the resistance offered by the membrane of the spore, the *Paramaecium* expels them just as it would any other

[1] *Ann. de l'Inst. Pasteur*, Paris, 1890, t. IV, p. 148.

[2] "Leçons sur la pathologie comparée de l'inflammation," Paris, 1892, p. 24; "Lectures on the comparative pathology of inflammation," authorised translation into English, London, 1893, p. 20.

excrementitious matter. The Infusorian behaves in the same way in regard to bacterial endospores.

Hay bacilli, which occur so commonly in the infusions in which the *Paramaecia* live, are digested in the endoplasmic vacuoles of the latter, but the spores of these bacilli, after a more or less prolonged sojourn in the vacuoles, are expelled with the excrement.

As by far the greater part of the body of a Protozoon is made up of digestive protoplasm, it is natural that infective epidemics should be very rare among these animalcules. The Infusoria and Rhizopods, organisms specially well adapted to live upon the lower Algae and Bacteria, are, practically, never subject to bacterial diseases. The infections observed in the Protozoa are due in most cases to the invasion of the lower Fungi, such as the *Chytridia*, the Microspheres, the *Saprolegniae* or the special organisms mentioned as occurring in the nuclei of *Paramaecia*. Further, these infections are met with most frequently in Protozoa which are incapable of carrying on true intracellular digestion or which are in the encysted stage, at which period the Infusoria, leading a passive existence, neither absorb nor digest nutriment. As an exception to the above general statement I ought to mention the epidemic in *Amoebae* caused by the *Microsphaera*[1] and the disease in *Actinophrys* observed by K. Brandt[2] and attributed to Fungi allied to the genus *Pythium*. In these two instances we have to do with parasites which live and develop in the interior of the active protoplasm of these Protozoa. Certainly a proportion of the parasites are expelled with the excrementa ; but there remain others which instal themselves in the protoplasm, multiply there and cause the death of their hosts. In these cases the digestive action of the protoplasm must be neutralised or paralysed by the secretions of the parasite. This aspect of the question, however, has so far not been considered.

In addition to intracellular digestion and the expulsion of parasites by the excretory function, the resistance offered by Protozoa to infective diseases should, in part, be ascribed to their great irrita-
[21] bility. Anyone who will watch the manœuvres of *Amoebae* or of certain Infusoria in the midst of a rich microscopic flora and fauna, will at once be struck by the preferences which these Protozoa exhibit in the choice of their food. *Amoebae* are often seen making search for Diatoms only, disdaining all other Algae, or again

[1] " Leçons sur la pathologie comparée de l'inflammation," p. 21 ; English edition, p. 17.
[2] *Monatsber. d. Berl. Akad. d. Wissensch.*, 1881, p. 388.

they may single out one species of Palmellaceae from a very varied flora. The Infusoria also have their likes and dislikes in the matter of food. Many of the ciliated Infusoria choose Bacteria to the exclusion of almost everything else; others, as *Nassula*, have a special partiality for the *Oscillariae*. A most striking example of this is afforded in *Amphileptus claparedei*, a voracious Ciliate, which chooses *Vorticellae* to the exclusion of all other animalcules; these it devours, and then becomes transformed into a cyst upon the peduncle of the *Vorticellae* it has devoured. This irritability clearly must control and guide the Protozoa in their relations with other organisms and enable them to escape the invasion of parasites.

In this connection I must mention a very interesting observation made by Salomonsen[1] and communicated to the Paris International Medical Congress in 1900. He was able to demonstrate the fact that almost all the ciliated Infusoria, on becoming aware of the proximity of dead bodies of kindred organisms, rapidly draw away, thus manifesting a very marked negative chemiotaxis. This property must, it is evident, protect them from any contamination by the parasites contained in the bodies of Infusoria that have succumbed to infective diseases.

We have, then, quite a number of facts which throw light on the natural immunity of the Protozoa against the action of pathogenic micro-organisms. Up to the present, however, we know nothing concerning the existence or the possibility of an acquired immunity among the lower animalculae against infective diseases. We are better informed as to the resistance of unicellular organisms to the action of soluble poisons, which is, in general, much more easily studied than is immunity against the micro-organisms themselves.

As a very large number of the higher animals are sensitive to the toxic action of poisons of bacterial origin, the question has been put, "May not the Infusoria also be poisoned by these micro-organismal products?" With the object of answering this question Gengou[2] has studied the influence of the toxins of tetanus and diphtheria on [22] the ciliated Infusoria. He was unable, however, to bring forward proof that these substances exert any special toxic action on the *Paramaecia*. These Infusoria withstand, perfectly well, doses of

[1] *Compt. rend. du Congrès internat. de Méd. tenu à Paris en* 1900. Section de bactériologie et de parasitologie.

[2] "Sur l'immunité naturelle des organismes monocellulaires contre les toxines" *Ann. de l'Inst. Pasteur*, Paris, 1898, t. XII, p. 465.

cultures of the diphtheria and the tetanus bacillus grown in broth and deprived of the bacilli by filtration as large as those of ordinary broth alone in which no bacilli have been cultivated. Gengou argues from this that the *Paramaecia* possess a natural and absolute immunity against these two toxins. When we take into consideration the fact that these poisons act but feebly at ordinary temperatures and are often innocuous to "cold-blooded" animals we may perhaps be tempted to attribute the immunity of the Infusoria to the temperature that was maintained in the incubator whilst Gengou's experiments were being carried on. Led by this train of thought Mme Metchnikoff tried the action of the blood-serum of eels, which is very toxic, not only for warm-blooded Vertebrates but also for cold-blooded Vertebrates and the Invertebrates, on the *Paramaecia*, and this at a low or medium temperature. This eel's serum, however, exerted no greater toxic action than did the blood-serum of other animals.

The microbial toxins are innocuous not only to the ciliated Infusoria but also to many other unicellular organisms. It is now well recognised that these toxins, exposed to the air, are soon inhabited by quite a rich flora of micro-organisms, amongst which Bacteria and Yeasts predominate. I have been able to prove[1] that these organisms are not only unaffected in their normal life by the presence of the toxins of diphtheria or tetanus but that they rapidly bring about the more or less complete destruction of these poisons. Gengou, also, observed that yeasts thrive luxuriantly in these bacterial toxins. The rapid increase of micro-organisms and the destruction of these poisons take place at temperatures varying from 15° to 37° C.

Whilst the lower organisms are refractory to bacterial toxins which in quite small doses are capable of killing man and the higher animals, many micro-organisms manifest a special sensitiveness to certain fluids of animal origin. In a succeeding chapter we shall treat at greater length of this microbicidal property of the humours. Here it is merely necessary to indicate certain facts concerning this property, regarding them solely from the point of
[23] view of the immunity of the lower organisms. The most striking example of the bactericidal power of an animal fluid is certainly that afforded in the action of the blood-serum of the rat on the anthrax bacillus. This fact, discovered in 1888 by von Behring[2], led

[1] *Ann. de l'Inst. Pasteur*, Paris, 1897, t. XI, p. 801.

[2] "Ueber die Ursache der Immunität von Ratten gegen Milzbrand," in the *Centralbl. f. klin. Med.*, Bonn, 1888, no. 38.

to the conclusion that the blood of the rat contains an organic base capable of killing and dissolving a considerable number of anthrax bacilli. Several observers have confirmed von Behring's observation and have supplemented it by the fact that the bacillus can be readily accustomed to the toxic action of this serum. Thus Sawtchenko[1], in an investigation carried out in my laboratory, was able, by successive cultures, to accustom the anthrax bacillus to an existence in the pure serum of the rat. In this case, therefore, there has been produced a real acquired immunity of a lower plant against a toxic substance of animal origin. More recently Danysz has demonstrated the same thing and has added several other facts which seem to throw light upon the means by which the bacterium becomes adapted to the poison. He has shown, in a work carried out in the Pasteur Institute[2], that the anthrax bacillus protects itself against the toxic action of the serum by surrounding itself with a thick sheath composed of a kind of mucus which fixes the toxin of the rat's blood and renders it harmless. This same mucus, but in smaller quantity, is likewise produced in a culture of the bacillus grown in ordinary broth. When such a culture is freed from the contained bacilli by filtration through porcelain and a little of this fluid is added to the rat's serum, this latter becomes less bactericidal than is a mixture of the same serum with ordinary broth. Danysz suggests that this is to be explained by the presence in the filtrate of a certain quantity of the mucous substance produced by the bacillus, which fixes and neutralises a portion of the "rat toxin." If, in place of sowing the ordinary bacillus, sensitive to this toxin, we inoculate the broth with an anthrax bacillus which has previously been accustomed to the rat's serum, we find that the liquid of this culture when filtered neutralises a larger proportion of the toxin. Danysz concludes from this that the acclimatised bacillus has acquired the property of producing more mucus than does the ordinary bacillus and that, for this reason, a greater quantity of this protective substance passes into the fluid of the culture.

The formation of a transparent sheath has several times been [24] observed in the anthrax bacillus, notably in cases where this organism happens to be in "a state of defence" against various noxious influences. For example, this sheath is well developed in the anthrax bacillus which invades the blood of lizards, animals which

[1] *Ann. de l'Inst. Pasteur*, Paris, 1897, t. XI, p. 872.
[2] *Ann. de l'Inst. Pasteur*, Paris, 1900, t. XIV, p. 641.

are in general very resistant to anthrax[1]. Under analogous conditions the streptococci which, as a rule, do not produce a mucous sheath, will develop one of exceptional size. The guinea-pig is in general very resistant to the streptococcus against which it exhibits a very effective reaction. Sometimes, however, this immunity gives way; in such instances, as demonstrated by J. Bordet[2], the streptococcus, in order to overcome the natural resistance of the guinea-pig, is found to have surrounded itself with a sheath of a thickness such as is seldom to be met with in the world of bacteria (Fig. 2).

Fig. 2. Streptococcus surrounded by a protective envelope.

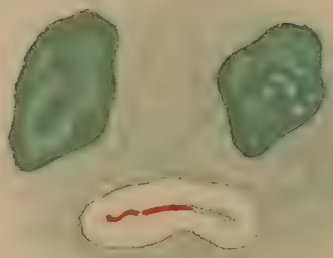

Fig. 3. Tubercle bacillus surrounded by a transparent envelope and enclosed in the giant cell of a gerbil.

Analogous facts are also observed in cases where the micro-
[25] organism is defending itself against the action of substances enclosed in animal cells. I may cite as an example the tubercle bacillus in the interior of the giant cells of a gerbil (*Meriones shawii*), where, under the influence of noxious substances contained in these cells, the tubercle bacillus (Fig. 3) envelops itself in a transparent sheath similar to that of the bacillus or of the streptococcus. As the action of the giant cell still does not cease, the tubercle bacillus secretes a second sheath (Fig. 4) and continues to surround itself with

[1] Metchnikoff, *Virchow's Archiv*, 1884, Bd. XCVII, S. 510.

[2] "Contribution à l'étude du sérum antistreptococcique," *Ann. de l'Inst. Pasteur*, Paris, 1897, t. XI, p. 177, Planche V.

quite a series of such envelopes (Fig. 5), thus coming to resemble a palmellaceous Alga surrounded by successive layers of membranes or certain other vegetable cells whose principal means of defence against all kinds of injurious influences consists in the production of these protective membranes.

Fig. 4. Another tubercle bacillus surrounded by two membranes.

Fig. 5. Tubercle bacillus surrounded by a series of concentric layers.

Quite recently Trommsdorf[1], in Buchner's laboratory in Munich, has carried out a series of experiments on the adaptation of the cholera vibrio and of the typhoid bacillus to the bactericidal substance found in the blood of the rabbit. He has been able to confirm the results of his predecessors and by various experiments has convinced himself that these two micro-organisms are capable of adapting themselves to existence in the defibrinated blood and in [26] the blood-serum of the rabbit.

The immunity, or acclimatisation of injurious organisms to different toxins, presents an undoubted analogy to the phenomena of adaptation shown by these organisms to mineral or organic poisons. It has long been known that the same species of Protozoa are met with in both fresh and salt water and that it is possible to gradually accustom Infusoria and *Amoebae* to tolerate an amount of sea salt which at first is absolutely fatal to them. This toleration is not acquired unless care be taken to increase the amount of salt very gradually: too abrupt a rise inevitably causing death. By this means Cohn[2] accustomed the

[1] *Arch. f. Hyg.*, München u. Leipzig, 1900, Bd. xxxix, S. 31.

[2] "Entwickelungsgeschichte der mikroskopischen Algen und Pilze," *Nov. Acta Acad. Caes. Leop. Carol.*, 1854, t. xxiv, p. 1.

fresh-water *Euplotes* to a life in artificial sea water containing 4 % of sodium chloride. In Balbiani's experiments[1] the fresh-water Monads (*Menoidium incurvum* and *Chilomonas paramaecium*) died very quickly on the addition of ½ % of this salt; but when it was added in small successive doses (0·05 per day), they readily became accustomed to a concentration of 1 %. In the encysted state the Protozoa are even more resistant than in the active state to the different salts that may be added to their normal culture medium. It is probable that the wall of the cyst interferes with the penetration of these substances into the endoplasm. If a small quantity of an aniline dye be added to a fluid containing encysted Infusoria, it is seen that the cyst-membrane becomes very intensely coloured but the body of the Infusorian remains unstained. The membrane absorbs a large amount of colouring matter, after which, being saturated, it ceases to take it up; but it does not allow the dye to penetrate into the endoplasm.

Balbiani (*loc. cit.* p. 580), having compared the action of the salts of sodium with that of the salts of potassium and lithium on Infusoria, comes to the conclusion that the injurious influence of these substances can only be partially explained by osmotic phenomena. In addition to these a purely chemical action must be invoked. He bases his opinion on the fact that the isotonic solutions of the three
[27] salts acting on Infusoria of the same species and same origin exert a different influence. The salts of potassium and of lithium act in a much more energetic fashion than do the sodium salts. Consequently, the Protozoa are able to adapt themselves progressively not only to noxious influences of a physiological character but also to those of a chemical nature. Thus Infusoria and Rhizopods can be accustomed to the action of high temperatures, to an intense light, etc. On the other hand they can also be habituated to the toxic actions of true poisons. Davenport and Neal[2] have established the fact that Stentors kept for two days in a weak solution of corrosive sublimate (0·00005 %) acquire a tolerance to a dose of this poison four times as great as the lethal dose for individuals previously kept in pure water. The same thing has been observed in connection with the toxic action of quinine. This immunity cannot be attributed to the selection and persistence of those Infusoria which possess a natural resistance to the sublimate.

[1] "Action des sels sur les infusoires," *Arch. d'anat. microsc.*, Paris, 1898, t. II, p. 595.

[2] "On the acclimatisation of organisms to poisonous chemical substances," *Arch. f. Entwickelungsmech.*, Leipzig, 1895, Bd. II, S. 564.

It is really acquired as the result of a direct and gradual chemical influence on the protoplasm of the Stentors which, once adapted, all survive doses which are lethal for the unacclimatised control organisms.

The vegetable micro-organisms, which are much more easily cultivated than are the Protozoa, frequently manifest most characteristic phenomena of acclimatisation. The first systematic researches in this direction were carried out by Kossiakoff[1] in the laboratory of Duclaux. He studied the antiseptic action of borax, of boracic acid, and of corrosive sublimate on the anthrax microbe and several other bacilli (*Bacillus subtilis, Thyrotrix scaber* and *T. tenuis*). He found that all these micro-organisms can be gradually accustomed to doses which are absolutely bactericidal to the same species when not so acclimatised. The acclimatised *Thyrotrix tenuis* withstands almost double the amount of bichloride of mercury that the non-acclimatised bacillus will resist. The ordinary anthrax bacillus will not develop at all if the culture medium contains more than 0·005 of boracic acid whilst the same organism, when accustomed by passage through successive cultures in which this substance is present in gradually increasing proportions, grows well in spite of the presence of 0·007 of the same antiseptic. Since these observations were made similar facts have been demonstrated by several other observers, and the ready acclimatisation of Bacteria to poisons is now generally admitted. Danysz [28] (*loc. cit.*), with the object of elucidating the mechanism of this adaptation, has studied the action of arsenic acid on the *Bacillus anthracis*. He has demonstrated that this bacillus will gradually accustom itself to grow in broth containing a quantity of arsenic acid which at first inhibited all development. During this phenomenon of adaptation, which is acquired after a series of passages through media more and more highly arsenicated, the bacillus secretes a coating of mucous substance which protects the sensitive parts of the microbial cell. Here, therefore, is formed something exactly corresponding to what the same observer has demonstrated in anthrax bacilli that have acquired a tolerance for rat's serum. This analogy extends even to the throwing out of the protective substance into the culture fluid. When one sows an ordinary unadapted bacillus in arsenicated broth to which has been added some of the fluid from a culture of the adapted bacillus, development takes place in a marked fashion. On the contrary when the same material is "seeded" into arsenicated

[1] *Ann. de l'Inst. Pasteur*, Paris, 1887, t. I, p. 465.

broth of the same composition but to which has been added the filtrate from an unadapted culture, the bacillus does not develop nearly so well. The difference is explained by the presence, in the fluid in which the adapted bacillus had grown, of a certain quantity of the mucous substance which fixes the arsenic and prevents it from acting on the protoplasm of the micro-organisms.

The Yeasts, also, adapt themselves very readily to antiseptics. This property has even had a practical application. We know that small doses of hydrofluoric acid are capable of preventing the proliferation of the yeast of beer, and Effront[1] has accustomed this plant to live in media containing an amount of hydrofluoric acid which is absolutely inhibitory to the unadapted yeast. Under these conditions the adapted cells undergo a stimulation which causes the production of a greater quantity of alcohol. The yeast, in adapting itself to antiseptic doses (300 mm. of hydrofluoric acid per 100 c.c. of beer wort), acquires a kind of immunity which it did not possess in the first instance. Moreover this acquired property can be hereditarily transmitted to new generations developed in ordinary beer wort to which hydrofluoric acid has not been added. The stimulating action of this substance on the fermentative property does not depend upon the acid reaction of the hydrofluoric acid, for other acids which are
[29] non-antiseptic, such as tartaric acid, are incapable of inducing it.

The acquired immunity against hydrofluoric acid is strictly specific, the yeasts that have been adapted to this substance becoming even more susceptible to the action of other poisons.

Duclaux[2] has already insisted on the relations which exist between antiseptics and foods. Formic aldehyde which has a very powerful coagulative and therefore strongly antiseptic action on protoplasm may actually serve as a food for micro-organisms. The *Thyrotrix tenuis*, studied in this connection by Péré[3], adapts itself to the presence of this aldehyde and utilises it for its nutrition. Here is produced something that recalls the case of the Protozoa that digest parasitic organisms.

It is now a current idea in microbiology that Bacteria and Yeasts which primarily do not make use of certain substances, adapt themselves to use them as nutrient substances. Dienert[4] has published a detailed work on the adaptation of the yeasts to milk-sugar. This

[1] *Monit. scient. du Dr Quesneville*, 1890, 1891, 1892, 1894.
[2] "Traité de Microbiologie," Paris, 1898, t. I, p. 238.
[3] *Ann. de l'Inst. Pasteur*, Paris, 1896, t. X, p. 417.
[4] *Ann. de l'Inst. Pasteur*, Paris, 1900, t. XIV, p. 139.

sugar is usually disdained by the yeasts that set up the fermentation of glucose; it is not difficult, however, to adapt them to galactose which they then attack and transform into alcohol and carbonic acid.

The Protozoa can be progressively accustomed not only to poisons but also to altered physical conditions. Thus, Dallinger[1] succeeded in raising the temperature of the water in which flagellated Infusoria were growing from 15°·5 to 23° C. without causing their death. By prolonging the experiment over several months, he was even able to habituate them to an existence at a temperature of 70° C. In the opinion of Davenport[2], a view which is shared by many other observers, this resistance to high temperatures was dependent on the abstraction of water from the protoplasm. Dallinger has also observed that in Infusoria that are accustomed to life in hot water, the vacuoles become smaller and smaller and may even actually disappear.

This adaptation, then, is a property that is very general and widespread in the microcosm of the unicellular organisms. It is connected with the intracellular digestion of solid food and with the absorption and transformation of soluble substances. These phenomena, chemical in character, are intimately linked with the irritability of microscopic organisms, which represents one of the fundamental properties of [30] living organisms.

A Protozoon, which is refractory to a parasite, may protect itself by flight or it may devour and digest the parasite; another, which acquires a tolerance in regard to a toxin or to a mineral poison, absorbs, fixes and transforms this substance. Consequently, in all these instances of immunity there is a reaction of the living elements of the organism, this being a direct consequence of the irritability of the protoplasm.

Before an Infusorian retreats from the dead body of an allied organism, before a Protozoon secretes a digestive fluid around the prey it has ingested, before a Bacterium secretes a glairy layer for its defence, etc., these unicellular organisms must receive sensations which provoke the above-mentioned reactions. It is to a celebrated botanist, Pfeffer, that we owe the most important researches on this irritability of unicellular organisms, researches which may be summed up in the general statement that this property is subject to the psychophysical law of Weber-Fechner. Pfeffer, by the observation of the

[1] *Journ. R. Micr. Soc.*, London, 1880, III, p. 1.

[2] Davenport and Castle, *Arch. f. Entwickelungsmech.*, Leipzig, 1895, Bd. II, S. 227.

movements of Bacteria under the influence of increasing stimulations, has established the fact that, conformably to this law, when the stimulus increases in geometrical ratio, the irritability increases in arithmetical ratio, that is to say, the reaction is proportional to the logarithm of the stimulation. In order that a motile bacterium (*Bacterium termo*), grown in a peptonised solution, may perceive a difference of medium, it is necessary to place it in a peptone solution of five times the original concentration; weaker solutions, in which the concentration is but three or four times greater than the original fluid, do not attract the bacteria at all; consequently these differences are below their chemiotactic sensibility.

The different reactions that are exhibited in the immunity of unicellular organisms, reactions which are dependent on the irritability of their protoplasm, therefore, come undeniably under the category of purely cellular phenomena.

CHAPTER II [31]

IMMUNITY IN MULTICELLULAR PLANTS

Infective diseases of plants.—Plasmodia of the Myxomycetes and their chemiotaxis.—Adaptation of the plasmodia to poisons.—Pathogenic action of *Sclerotinia* upon Phanerogams.—The cicatrisation of plants.—Defence in plants against Bacteria.—Sensitiveness of vegetable cells to osmotic pressure.—Adaptation of plants to modifications of osmotic pressure.—Dependence of the chemical phenomena upon the irritability of the vegetable cells.—The law of Weber-Fechner.

For several reasons this immunity in the vegetable kingdom cannot be treated in a satisfactory fashion. Much attention has been devoted to the pathology of plants and the etiology of a number of vegetable diseases was well established at a period when we were still groping in the dark for the causes of infective diseases in man and the higher animals. In spite of this, the botanist has relegated the study of the phenomena of immunity to a secondary position, and up to the present no work specially devoted to this subject has appeared. It is only incidentally that the question of the resistance of certain plants to morbific factors capable of infecting or intoxicating them has been touched upon. We should require, therefore, to carry out special researches in this direction and to make a very complete study of botanical literature, before we should be able to present to our readers a *résumé* of the question of immunity in the vegetable kingdom. Such a programme being impossible we must content ourselves with borrowing from the botanists certain facts which throw light on some aspects of the general problem in which we are interested.

Many of the higher plants are subject to infective diseases set up by the lower plants, of which the most important are the Fungi. Whereas in the animal kingdom the majority of the infections are due to Bacteria, these micro-organisms rarely occur in plants ; [32] moreover when they are present the part they play is nearly always a secondary one. This difference is due mainly to the chemical

composition of the "humours" in the two kingdoms, the cell-juice of plants being generally acid; under this condition the Fungi develop much better than do the Bacteria.

The various modes of defence against infective diseases that have been met with in unicellular organisms are also found in the multicellular plants. Whereas in almost all plants the cells are rigid, owing to the presence of a well-developed membrane, some of the lower plants have preserved a condition in which the protoplasm is completely naked and capable of movement. Myxomycetes are specially distinguished by an amoeboid stage of existence and by the formation of large plasmodia which protrude protoplasmic processes and exhibit a kind of locomotion similar to that met with in the Rhizopods and the Sporozoa.

Infective diseases among the Myxomycetes must be very rare since, up to the present, they have not been noted by a single observer. It is very probable that the plasmodia get rid of the infective germs, as do the Protozoa, both by expulsion of the parasites and by means of intracellular digestion. This latter takes place in a medium which is distinctly acid and by means of a soluble ferment described by Krukenberg[1] as a kind of pepsin. I need not here enter into further detail as I have already treated this subject in my *Lectures on the comparative pathology of inflammation*. The fact that the Myxomycetes can ingest living organisms has been demonstrated by Celakovsky, jun.[2], who has observed that the spores of the various Fungi can germinate in the interior of the plasmodium. Whilst our conceptions concerning the resistance of the plasmodia in regard to micro-organisms are merely based upon analogies and hypotheses, our ideas as to their immunity against soluble substances rest on well-established experimental facts. We owe to Stahl[3] our first information as to the mode by which the plasmodia resist poisons. When they are placed in contact with solutions of salts, of acids or of sugar in a sufficiently concentrated form to bring about an injurious action, the plasmodia make use of their amoeboid power of motion to escape
[33] from these fluids. Hence they exhibit a *negative chemiotaxis*, exactly parallel to that so often observed in the case of the unicellular organisms. Consequently there is in the Myxomycetes a natural immunity due to the activity of their movements. Further, a kind

[1] *Untersuch. a. d. physiolog. Inst. d. Univ. Heidelberg*, 1878, Bd. II, S. 273.
[2] *Flora*, Marburg, 1892, Bd. LXXVI, S. 182.
[3] *Botan. Ztg.*, Leipzig, 1884, S. 161.

of acquired immunity in these plants has also been demonstrated by Stahl. The following is the passage in his paper referring to this subject, a passage very important from a general point of view[1]: "If we replace the water in a vessel by a 1 or 2 % solution of glucose, we observe either the death of the plasmodia, if the action is too rapid, or merely their retreat from the glucose solution. Even solutions of ½ or ¼ % are at first avoided by the plasmodia and, should the action be too rapid, may cause their death. Usually the plasmodia emigrate into those portions of the substratum remote from the solution, to return after some time, often only after several days, and immerse themselves in the solution of glucose as they do in an infusion of tan, although with more hesitancy. *Consequently the Myxomycetes accommodate themselves slowly*[2] to a more concentrated solution, probably by giving up a certain proportion of their water. I was able to observe the same phenomena with even much more concentrated solutions (2 %). A plasmodium which at the end of several days had adapted itself to a 2 % solution of glucose and had sent out numerous processes into it, found itself injuriously affected when the sugar solution was suddenly replaced by pure water. Those that remained alive had retired to a great distance from the upper layer of the fluid and did not descend again until the end of the second day. After a fresh change of fluid we were able to observe first the repulsion and later the attraction of the plasmodia, but a certain time always elapses before the plasmodia become accustomed to the change in concentration. We obtain the same result when we replace a 2 % solution, not by pure water, but by a ½ or a 1 % solution" (p. 166).

De Bary[3] had already interpreted these facts as being due to an immunity acquired by the plasmodia, the result of an adaptation of these organisms to solutions which they had, at first, carefully avoided. He threw out the suggestion that a similar adaptation might take [34] place in regard to solid substances ingested by the Myxomycetes.

As these phenomena of acquired immunity, in organisms so primitive and of so simple a structure, are of immense importance from the point of view of Immunity in general I felt bound to submit them to a personal investigation. I found it an easy matter to

[1] [Stahl used plasmodia which had spread themselves on a substratum of wet filter paper applied to the inside of glass vessels, its lower edge touching the surface of the experimental fluid at the bottom of the vessel (Translator).]

[2] The italics are M. Metchnikoff's.

[3] "Vergleichende Morphologie u. Biologie der Pilze, Mycetozoen u. Bacterien," Leipzig, 1te Aufl., 1884; also authorised English translation, Oxford, 1887.

accustom the plasmodia of *Physarum* to solutions of arsenious acid which at first repelled them in a very striking manner. This adaptation manifests itself by movements of the plasmodia and by the change from negative chemiotaxis (repulsion) to positive chemiotaxis (attraction).

It is impossible in the present state of our knowledge to state precisely the modifications that the plasmodia undergo during this process of adaptation. Stahl supposes that they depend "on some special properties of the plasmodia (probably in a greater or less richness in water)"; and that it is a case "not of simple phenomena, easy of explanation, but of extremely complicated phenomena of irritability."

It is evident that, in this case of acquired immunity, we have not to do with a question of physical or chemical modification of the solutions employed but solely with reactive phenomena on the part of the living plasmodia.

After a phase of active life, during which the Myxomycetes move, feed, digest and expel waste products as do the lower animals, there comes a stage when they become immobile and transform themselves into a number of sporangia filled with rounded spores. Before leaving their animal aspect for that of true plants, the plasmodia exhibit entirely new attributes. They reject all nourishment and no longer ingest foreign bodies; they avoid the moisture which previously attracted them and cease to shrink from the light.

Having come to maturity, the Myxomycetes declare themselves true plants and lead a passive life until the new generation comes forth. Most plants are restricted to this passive phase of the Myxomycetes. In these latter it persists only for a short period, whereas in almost all plants it is the permanent condition. It is at this stage that these organisms are liable to the attack of parasites against which it is necessary for them to oppose all their means of defence. Our knowledge of these means of defence is as
[35] yet, as I have already stated, very imperfect, and the example of *Sclerotinia libertiana* (or *Peziza sclerotiorum*) which has been the subject of the researches of de Bary[1] remains up to the present the one that has been most thoroughly studied.

This Fungus, belonging to the group of the Discomycetes, invades many species of plants and often produces great ravages amongst the cultivated plants of our fields and gardens, such as colza, hemp

[1] *Botan. Ztg.*, Leipzig, 1886, SS. 377, 393, 409, 433, 449, 465.

petunias, dahlias, etc. The mycelium of this *Sclerotinia* develops in the stems of herbaceous plants and produces sclerotia inside them, forms of resistance, which in this instance are black and resemble small particles of mouse excrement.

The spores of the *Sclerotinia* germinate and form mycelial threads on the surface of the plants. In order that they may penetrate into the tissues these threads must attack the cell-membrane and for this purpose they secrete a fluid, which contains both a digestive ferment and oxalic acid, the latter being essential for the action of the ferment.

The presence of this "toxin" has been demonstrated by de Bary by macerating the mycelium of the *Sclerotinia*. The resultant extract has a well-marked action on the tissues of many plants (carrot, Jerusalem artichoke, chicory, etc.). Under its influence the protoplasm of the cells contracts, a genuine plasmolysis is set up, the cell-membrane swells and its layers between the cells are dissolved. As the result of this digestive action, the cells become separated and the tissue softens. This extract, when heated to 52° C., loses its digestive action on the cellulose membrane, but still retains its power of setting up plasmolysis. This reaction to temperature confirms the view that the juice of the Fungus contains a soluble ferment. The results of de Bary's researches have been confirmed and in part supplemented by the experiments of Laurent[1].

It is a fact of common observation that the *Sclerotinia libertiana* invades for the most part young plants. It may therefore be asserted that the disease produced by this Fungus is, like scarlatina or measles in the human subject, an "infantile" disease. De Bary suggested that the immunity of adult plants must depend on the greater resistance which their cell-membranes offer to the fluid secreted by the mycelial filaments. Direct experiments have shown the accuracy of his suggestion. Whilst the fluid extracted from the *Sclerotinia* readily digests the tissue of young plants it leaves intact that of adult [36] plants of the same species.

In the course of this disease we have a struggle going on between two plants. The parasite brings into play toxic and digestive secretions with which it seeks to impregnate its host. The attacked plant defends itself by the secretion of membranes capable of resisting the action of the secretions of the Fungus. This struggle by means of chemical substances is, however, directed by the activity of the living

[1] *Ann. de l'Inst. Pasteur*, Paris, 1899, t. XIII, p. 44.

cells of the two belligerent plants, an activity dependent upon the irritability of their protoplasm.

The example we have just studied may serve as a type for our examination of the phenomena of immunity in the vegetable kingdom. The crux is above all to prevent the access of the parasites to the vital parts of the plant by opposing to them membranes as resistant as possible. Consequently the majority of plants, directly the smallest lesion is produced, react by an abundant cell-proliferation and by the suberisation of the outer layers. The cell-membranes of the latter thicken, the cellulose is transformed into suberin ; a layer of cork not very permeable to fluids and gases being thus formed. By suberisation the plant reacts against grosser lesions, incisions or burns, as well as against the decay set up by micro-organisms.

Massart[1], in an extremely interesting memoir, has brought together the known data concerning cicatrisation in plants and has demonstrated the fact that it is a very variable process. In many leaves after being damaged there is no attempt to react by forming cicatricial tissue. Many aquatic and marsh plants react but feebly. Their tissues die and turn brown, the plants failing to defend themselves by cicatrices, probably owing to the ease with which the lost parts can be replaced. When, however, in the same plants, there is produced a lesion of parts which are of great importance for the preservation of the integrity of the individual or a lesion of the organs which enable the plant to continue its existence through the winter, cicatrisation of the wounds takes place rapidly.

The old or adult parts in most cases react differently from the young parts. Thus, the young leaves of *Clisia* (the example selected by Massart) react to traumatism very promptly and form a genuine
[37] callus which makes good the injury, but the adult leaves merely produce a layer of cork in the immediate neighbourhood of the lesion.

The essential mechanism of cicatrisation has not yet been satisfactorily analysed, but it is evident, when all is said and done, that it is directed by the irritability of the living protoplasm of the vegetable cells.

Many plants protect their wounds with a kind of dressing, using for that purpose juices which harden on exposure to the air. Sometimes these juices, *e.g.* latex, are preformed in the plant and are as it were always ready for use ; at other times they may be formed only

[1] "La cicatrisation chez les végétaux," *Mém. couron. de l'Acad. roy. de Belgique*, Bruxelles, 1898, t. LVII.

as the result of an injury. In this latter case the resins and gums which serve to close the wound and to protect the living parts receive the name of "cicatricial secretions" (Wundsecrete). According to the view first formulated by de Vries, those juices which harden under the action of air prove of great service both as natural "dressings" and as safeguards against the attacks of plants and animals. Indeed many of these secretions contain essences whose antiseptic and toxic action is now generally recognised[1].

The suberisation, the formation of a callus, and the secretion of juices which close the wounds, are all means readily utilised and very potent in ensuring the resistance of plants against all sorts of injurious influences which may be set up by a morbid condition. But these processes are not the only means which plants have at their disposal. The living elements of plants usually secrete a cell-juice of acid reaction which plays a very important part in the defence of plants against pathogenic agents. Laurent[2] has studied this phase of the immunity of plants against bacterial decay. A variety of the *Bacillus coli communis*, according to this observer, attacks the potato by means of its secretions in a fashion analogous to that already described when discussing *Sclerotinia*. This bacillus produces a soluble ferment which has the power of digesting the cellulose membrane in the tuber of the potato, and at the same time secretes an alkaline juice without which this digestion cannot go on. Heating to 62° C. destroys the soluble ferment and the fluid thus heated is no longer able to digest the layers of the cell-membrane between the cells. In spite of exposure to this temperature, however, it still retains intact one or even several substances which may continue to cause con- [38]
traction of the protoplasm and ultimately kill it.

When Laurent placed cut halves of tubers coming from races of potato which were most resistant to bacterial decay in the fluid produced by the *Bacillus coli* and afterwards inoculated them with the bacillus itself, he invariably found that the vegetable cells were profoundly affected.

The alkaline secretions of the bacillus studied by Laurent may be neutralised by the acid juice of the potato, and when certain races of tubers prove immune from decay, it is, according to this observer, because of the production of sufficiently acid cell-juices. Moreover he

[1] Cf. Frank, "Die Krankheiten der Pflanzen," Breslau, 2te Aufl., 1895, Bd. I, S. 43.

[2] "Recherches expérimentales sur les maladies des plantes," *Ann. de l'Inst. Pasteur*, Paris, 1899, t. XIII, p. 1.

actually succeeded in communicating an artificial immunity to varieties of the potato which were most susceptible to decay by immersing them for several hours in solutions of certain organic acids. On the other hand, when he treated varieties endowed with a well-marked natural immunity with alkaline solutions, the tubers became very susceptible to the decay set up by the bacillus.

The struggle between the potato and the *Bacillus coli* reduces itself, then, to the chemical reaction between the alkaline cell-secretions of the micro-organism and the acid secretions of the potato. This general fact, according to Laurent, explains the part played by certain manures in determining the susceptibility or the resistance manifested by the potato and many other plants against infective diseases.

We know that the addition of phosphates to the soil increases the immunity of certain cultivated plants. These substances are greedily absorbed by the roots and produce acid salts which are dissolved in the cell-juice. The nitrogenous manures, on the other hand, both potassic and lime, diminish the resistance of the same plants, probably from the fact that they bring about a diminution of the acidity of the cell-juice.

But these manures can act differently on different plants. Thus the same phosphates which confer immunity on the potato against bacterial decay render the Jerusalem artichoke more susceptible to the attacks of the *Sclerotinia*.

Laurent explains this fact as due to the difference in the reaction of the medium, which favours the action of one or the other of the
[39] soluble ferments of the two parasites. The ferment of the bacillus digests the cell-membrane in an alkaline or feebly acid medium, whereas the hyperacidity which results from the absorption of the phosphates prevents this digestion and consequently aids the plant in its struggle. On the other hand, the ferment of *Sclerotinia*, as is seen from the researches of de Bary, will digest cellulose even in a distinctly acid medium. The hyperacidity, induced by the phosphated manure, in this case favours the parasite and enables it to gain the upper hand in the struggle with the tissues of the artichoke.

In addition to neutralising the microbial products the acids of the cell-juice also act injuriously on most bacteria, which will only develop in neutral or alkaline media; it is for this reason that bacterial diseases are so much rarer in plants than in animals.

The secretion of cell-juices is consequently a very important

element in the defence of plants; it will be useful, therefore, to ascertain as definitely as possible the essential mode of its action. Vegetable cells are as a rule very sensitive to the influences to which they are exposed; they distinguish with great precision the changes which take place in their surroundings. They are, indeed, capable of discerning not only the physical properties but also the chemical composition of the medium in which they live.

Vegetable cells estimate very accurately the osmotic pressure of the fluid which bathes them, and they react towards this solution by increasing or diminishing their own internal pressure. Van Rysselberghe[1], in an investigation very carefully carried out, demonstrated that when vegetable cells (especially the epidermic cells of certain species of *Tradescantia*) are placed in a solution of greater density than that to which the cells are accustomed, the intracellular pressure increases; in a solution of less density the pressure diminishes. These changes in osmotic pressure are due to variations in density of the cell-juice, whilst these variations are in turn determined by chemical transformations. Thus, if the cell be treated with a too concentrated solution it produces oxalic acid, which dissolving in the cell-juice, is, owing to the smallness of its molecule, very osmotic.

With the purpose of confirming this by exact facts van Rysselberghe has studied the acids of the cell-juice of *Tradescantia*. In the normal [40] juice he found that malic acid was constantly present and, in rare cases only, traces of oxalic acid. He then determined the acids present in the leaves of the same plant after they had been several days in contact with fairly concentrated solutions of cane sugar. In each analysis he found oxalic acid in quite appreciable quantity. There is then, in the plant which adapts itself to more concentrated solutions of the medium, a production of oxalic acid which serves the purpose of increasing the pressure of the cell-juice.

The origin of this oxalic acid could not be accurately demonstrated, but van Rysselberghe considers that it is probably formed at the expense of the glucose.

According to the researches of Giessler oxalic acid is localised specially in the epidermis and generally in the peripheral tissues of plants; it is very probable, therefore, that it fulfils a protective *rôle* against all kinds of injurious influences. Botanists hold indeed that oxalic acid keeps herbivorous animals, especially slugs and plant lice,

[1] "Réaction osmotique des cellules végétales," *Mém. couron. de l'Acad. roy. de Belgique*, Bruxelles, 1899.

from attacking plants that are rich in this substance. It is of use, also, in retaining the moisture in the superficial cells. It is very probable that it also plays an important part as a factor in the maintenance in plants of immunity against bacterial diseases.

The vegetable protoplasm, which is capable of increasing the production of acids in order to raise the osmotic pressure, can also, in case of need, cause a diminution.

When the cells of *Tradescantia* are transferred from a concentrated solution into one much more dilute there may often be observed a precipitation, in the cell-juice, of crystals of oxalate of lime; this brings about a diminution in the osmotic pressure. When the density of the medium is altered, and the vegetable tissue is again transferred to a stronger solution, the oxalate crystals are seen to dissolve, as a result of a new production of acid.

These chemical processes, so important to the life of plants in general and for ensuring to them immunity against infective agents in particular, are dependent upon the irritability of the protoplasm. Imprisoned in its resistant and more or less thick membrane, the living part of the vegetable cell estimates with nice discrimination every change that takes place around it.

[41] Massart[1] has shown that the stimulation produced by traumatism is often propagated a considerable distance and may excite a reaction in very remote cells. If the mid-rib of a leaf of *Impatiens sultani* be cut near the base of the limb the wound does not cicatrise but, a few days later, the leaf becomes detached from the stem.

Irritability is a fundamental property of all living beings. The plant may react by rapid movements, as in the case of the *Mimosa pudica*, or more slowly—by chemical reactions—as in the case of adaptation to density of medium. These reactions are produced as the result of various irritabilities which exhibit a specific character.

It is this specificity that determines whether the reaction that is manifested by the movements shall be produced in this direction or in that. The stem, owing to the specific irritability of its living parts, turns to the light; whilst the root, guided by a different irritability, grows down into the soil.

The irritability of plants, like that of unicellular organisms, is subject to the psycho-physical law of Weber-Fechner. Pfeffer[2] first

[1] "La cicatrisation," *l.c.*, p. 61.

[2] *Untersuch. a. d. botan. Inst. zu Tübingen*, Leipzig, 1884, Bd. I, S. 363.

demonstrated this for the motile spermatozoids of the Cryptogams. Massart[1], by a series of ingenious experiments on the irritability of a Mould (*Phycomyces nitens*) to light, has shown that the same law regulates the movements of this plant towards the source of light. This irritability of the Fungus to light is much more delicate than is the chemiotaxis of the spermatozoids of the Mosses and the Ferns and than that of the Bacteria.

Errera concluded from a consideration of the experiments of van Rysselberghe that the osmotic reaction of plants must also come under this psycho-physical law. His pupil at his request made systematic researches on the subject and the results have entirely confirmed his prevision. According to the data obtained by van Rysselberghe[2], the cellular osmotic reaction increases in arithmetical progression as the osmotic stimulation increases in geometrical progression. The osmotic reaction is therefore proportional to the logarithm of the stimulation.

To sum up, the phenomena of adaptation and of immunity in plants [42] are, as in the unicellular organisms, very widely distributed. Plants defend themselves by means of their resistant membranes and by secretions whose physical and chemical properties they are able to modify. These phenomena are dependent on the living parts of the cell which regulate them according to their greatly developed irritabilities. Thanks to this power, plants can gradually adapt themselves to concentration of the medium and to the presence of poisons which, at first, set up serious disturbances. Plants therefore, alongside a natural immunity, possess an acquired immunity against many pathogenic agents.

[1] "Recherches sur les organismes inférieurs," *Bull. de l'Acad. de Belgique*, 1888, 2e série, t. XVI, v, 12.

[2] *L.c.*, p. 40.

[43]

CHAPTER III

PRELIMINARY REMARKS ON IMMUNITY IN THE ANIMAL KINGDOM

Examples of natural immunity among the Invertebrates.—Immunity against micro-organisms and insusceptibility to microbial poisons are two distinct properties.—The refractory organism does not eliminate micro-organisms by the excretory channels.—It destroys them by a process of resorption.—The fate of foreign bodies in the organism.—The resorption of cells.—Intracellular digestion.—This digestion effected by the aid of soluble ferments.—Digestion in Planarians and Actinians.—Actino-diastase.—Transition from intracellular digestion to digestion by secreted juices.—Digestion in the higher animals.—Enterokynase and the part it plays in digestion.—The psychical and nervous elements in digestion.—Adaptation of the pancreatic secretion to the kind of food.—Excretion of pepsin in the blood and in the urine.

As shown in the two preceding chapters unicellular organisms and plants afford evidence of numerous phenomena of immunity. Alongside natural immunity we find in them undoubted evidence of an adaptation to the presence of morbific agents, evidence which warrants us in inferring that cases of acquired immunity are frequent. This being the case it is quite natural that the animal kingdom should be no exception to the general rule. Indeed, immunity against pathogenic agents is widely distributed in animals, and we continually see manifestations of natural immunity not only against parasites and their toxins, but against poisons in general. Just as frequently we find cases of acquired immunity against these morbific agents.

As yet we know but little concerning the phenomena of immunity in the lower animals belonging to the great group of the Invertebrata. But it may be affirmed with certainty that these also are often endowed with a natural immunity against micro-organisms and bacterial toxins. As an example I may cite the case of the large white larvae of the Rhinoceros beetle (*Oryctes nasicornis*) frequently met with in tanner's bark. Very susceptible to the cholera vibrio—$\frac{1}{8000}$ of a culture[1] of this organism being sufficient to set up a fatal

[1] [Probably a surface growth on a sloped agar tube (Transl.).]

septicaemia—these larvae exhibit a very remarkable natural immunity [44] against the bacilli of anthrax and diphtheria. A large dose of bacteria of the second anthrax vaccine, fatal to rabbits, guinea-pigs and mice, is borne without any inconvenience by the larvae of the Rhinoceros beetle. They are equally refractory to large doses of the diphtheria bacillus. And yet, there are not wanting species of insects which are susceptible to these same micro-organisms. Thus, according to A. Kovalevsky[1], crickets contract anthrax very readily even at moderate temperatures (22°—23° C.). On the other hand they are, according to the same author, refractory to the bacillus of avian tuberculosis. Many of the Invertebrata, studied from this point of view, present analogous facts, with which, however, we need not at present occupy ourselves.

In the Vertebrata in general and in Man in particular, natural immunity against many infective diseases and soluble poisons is so widespread that we are at no loss to find examples for citation. We have a whole series of human infections whose study is rendered particularly difficult simply because of the natural immunity of all other species of animals from these infections. Such are syphilis, scarlatina, leprosy, exanthematous typhus, etc. On the other hand, a large number of diseases, very infective for domestic animals, are quite innocuous to man. In this group we have cattle plague, strangles, contagious pleuro-pneumonia, fowl cholera, pneumo-enteritis of pigs, and a number of other diseases.

As in a very large majority of instances pathogenic organisms act through the agency of their toxic products, one might believe—and this has been assumed repeatedly—that natural immunity against infective diseases is dependent on the insusceptibility of the refractory organism to the specific poisons.

Such a supposition cannot survive criticism. We have undoubted instances of a species of animal being resistant both to a micro-organism and to its toxin. Such instances, however, are rare and usually an organism that is refractory or only slightly susceptible to the micro-organism itself is very susceptible to its toxic products. Even those micro-organisms which come almost constantly in contact with the human organism without becoming pathogenic, may produce toxins capable of gravely affecting health.

[1] " Etude expérimentale sur les glandes lymphatiques des invertébrés," *Mélanges biol. de l'Acad. d. sc. de St-Pétersb.*, 1894, t. XIII, p. 458.

[45] Let us take as an example the bacillus of blue pus. This organism is most widely diffused in human surroundings. According to Schimmelbusch[1] it is met with on the skin of the arm-pits and of the inguinal region of one-half of mankind. From the skin it very often passes into the dressings of wounds which then assume the characteristic and so long recognised blue colour. The same bacillus is also found in the intestines of both sick and healthy persons. Jakowski[2] has met with it in the faeces coming from intestinal fistulae in two women who had undergone operations. Now, in spite of these specially favourable conditions for the production of infection, the *Bacillus pyocyaneus* has remained harmless. It is only in children, and even then rarely, that it can be convicted of exciting disease. Man, then, usually enjoys a true natural immunity against the *Bacillus pyocyaneus*. And yet it is not to his insusceptibility to the pyocyanic toxin that he is indebted for this immunity. Schaffer[3], having injected himself in the shoulder with half a c.c. of a sterilised culture of *B. pyocyaneus*, developed fever and an erysipelatous swelling. Bouchard and Charrin[4] injected pyocyanic toxin into patients who reacted with more or less fever and by other toxic symptoms.

Another extremely common saprophyte, the *Micrococcus prodigiosus*, is incapable of setting up an infective disease, but this does not prevent its products from exercising a toxic action, often very grave, in man. The frog, which is refractory to the cholera vibrio, undergoes a fatal intoxication when cholera toxin is injected. One of the most striking examples is furnished in the case of the human tubercle bacillus and tuberculin. Man is much more resistant than is the guinea-pig to the pathogenic action of this organism, yet he is incomparably more susceptible to its toxin (tuberculin). According to the researches of Behring and Kitashima[5], the sheep, of all species of mammals, is most susceptible to the tubercular poison; the Bovidae and the guinea-pig occupy an inferior rank in the scale of susceptibility. On the other hand, the guinea-pig is very susceptible to the tubercle bacillus; the Bovidae are less so and the
[46] sheep is still more resistant to tuberculosis. It is unnecessary to multiply instances. Immunity against microbial infection and against

[1] "Ueber grünen Eiter," Volkmann's *Samml. klin. Vortr.*, No. 62, Leipzig, 1893.

[2] "Processus chimiques dans les intestins de l'homme," *Arch. d. sc. biol. de St-Pétersb.*, 1892, t. I, p. 539; *Ztschr. f. Hyg.*, Leipzig, 1893, Bd. XV, S. 474.

[3] Cited by Schimmelbusch, *l.c.*

[4] *Compt. rend. Acad. d. Sc.*, Paris, 1892, t. II, p. 1226.

[5] *Berl. klin. Wchnschr.*, 1901, S. 163.

intoxication are two distinct properties, so that it is impossible to reduce the former to an insusceptibility to toxins. We must therefore consider these two kinds of immunity separately and we will first consider the resistance of the animal organism against living infective micro-organisms.

Refractory human beings and animals may be inoculated with a large number of micro-organisms without being affected. Thus Opitz[1] injected 10,000,000 organisms into the blood of a dog. Twenty minutes later he could find no more than 9000. It is then quite natural to ask, What becomes of these micro-organisms after they have made their way into the interior of the refractory organism? It has been suggested that the animal gets rid of the pathogenic germs much as it does of all kinds of soluble poisons. Certain of these poisons, such as iodine and alcohol, are in great part eliminated by the kidneys ; others, such as iron, by the alimentary canal. Why, it is asked, should not micro-organisms also be eliminated by the same channels? Flügge has adopted this view and has expounded it in his work on ferments and micro-organisms[2]. Moreover he suggested to Wyssokowitch[3] that he should carry out a large series of experiments with the object of verifying this theory. But numerous very careful researches have given a result quite at variance with the forecast made by Flügge. Micro-organisms of various species, injected into the blood-vessels of rabbits and dogs, were, in those cases where these animals are refractory, never eliminated, either by the kidneys or by any other of the excretory channels which were studied. When bacteria pass into the secretions, lesions of the tissues, more or less grave, are invariably present.

This result has been repeatedly confirmed and has been accepted as a general experience. The elimination of micro-organisms by the urine indicates not merely the absence of immunity, but implies, also, a susceptibility of the organism. In many septicaemias, such as those produced by the anthrax bacillus, the streptococcus and other bacteria, or in less generalised diseases, such as typhoid fever, bacteria are found in the urine, often in large numbers. In these [47] cases it is a question of anything but a refractory condition even of the slightest degree.

[1] *Ztschr. f. Hyg.*, Leipzig, 1898, Bd. XXIX, S. 548.

[2] "Fermente und Mikroparasiten" in Ziemssen u. Pettenkofer's "Handbuch der Hygiene," Leipzig, 1883.

[3] "Ueber die Schicksale der in's Blut injicirten Mikroorganismen," *Ztschr. f. Hyg.*, Leipzig, 1886, Bd. I, S. 1.

In recent years, however, several works have been published the aim of which was to demonstrate the inaccuracy of this apparently well-established thesis. Biedl and Kraus[1] in Vienna took the initiative and announced in a detailed work that micro-organisms can readily pass intact into the kidney and that this organ in virtue of its physiological function eliminates them. The organisms were said to leave the blood capillaries by the normal process of diapedesis and were then eliminated with the urine. The liver in a physiological condition, according to the researches of these authors, is equally capable of allowing of the passage of micro-organisms ; indeed it aids in discharging them from the system. On the other hand, the pancreas and the salivary glands were incapable of fulfilling this function. Von Klecki[2] obtained similar results. He also holds that the kidney is the principal organ of elimination for micro-organisms which have penetrated into a refractory organism.

With these contradictions before him, Opitz[3] set himself to study this question in Flügge's laboratory at Breslau. Having critically reviewed the technical methods of his predecessors and carried out a series of new experiments, he declared categorically "that a physiological excretion, by the kidneys, of the micro-organisms which circulate in the blood, does not exist." For Opitz "the frequent appearance of micro-organisms in the urine of animals into whose blood, a short time previously, living bacteria have been injected, is due to mechanical and chemical lesions of the vessel wall and of the renal epithelia."

This question might be looked upon as definitely settled in favour of the first results obtained by Wyssokowitch were it not that other voices had been raised in favour of a physiological excretion of the micro-organisms by the renal channels. Pawlowsky[4] has recently published a long work on this subject in which he attempts to demonstrate that certain micro-organisms, even when introduced into the subcutaneous tissue of animals, pass very rapidly
[48] (at the end of a quarter of an hour) into the uropoietic organs and are eliminated with the urine.

It was necessary to put an end to these controversies and Métin[5] undertook a series of researches at the Pasteur Institute with the

[1] *Ztschr. f. Hyg.*, Leipzig, 1897, Bd. XXVI, S. 353.
[2] *Arch. f. exper. Path.*, Leipzig, 1897, Bd. XXXIX, S. 39.
[3] *Ztschr. f. Hyg.*, Leipzig, 1898, Bd. XXIX, S. 528.
[4] *Ztschr. f. Hyg.*, Leipzig, 1900, Bd. XXXIII, S. 261.
[5] *Ann. de l'Inst. Pasteur*, Paris, 1900, t. XIV, p. 415.

object of clearing up this question. He guarded himself against the objections justly made against his predecessors and conducted his experiments under unexceptionable conditions. He injected several species of micro-organisms into the veins of rabbits and into the subcutaneous tissue of guinea-pigs. At various intervals he performed laparotomy on these animals, pulled out the bladder and drew off the urine in such a fashion that no trace of blood could get into it. The results were most conclusive. Never, when the experiment was conducted under the rigorous conditions just mentioned, did the micro-organisms traverse the kidneys of resistant animals nor were they ever met with in their urine.

Métin's researches on the passage of micro-organisms through the liver in refractory animals gave the same results. In no case was he able to find in the bile any of the organisms that had been injected into the blood or under the skin. At the end of his memoir Métin sums up his results as follows: "(1) The kidneys and the liver are impermeable to bacteria introduced into the organism, subcutaneously or intravenously; (2) when the culture tubes contain colonies of the injected micro-organism, it is because there has been a certain amount of blood in the fluid inoculated, this being an indication of a vascular or epithelial lesion, either mechanical or chemical." We were present at M. Métin's experiments and can bear witness to their exactitude.

There can no longer be any doubt then on this point. The elimination of the micro-organisms from the refractory animal takes place, as indicated in Wyssokowitch's first investigation, neither by the kidneys nor by the liver. Some observers have asserted that this elimination may take place by the sudoriparous glands. Thus, Brunner[1] made experiments with young pigs and cats into which he had previously injected micro-organisms, for the most part pathogenic. Then producing a transpiration by means of pilocarpin, he "cultivated" the sweat and noted the development of the same bacteria as he had introduced into the blood. In a single experiment with a saprophyte (*Coccobacillus prodigiosus*) he obtained a positive [49]
result, from which he concludes that the refractory animal gets rid of bacteria which circulate in its blood by way of the sudoriparous glands. It is scarcely allowable to draw any conclusion from this experiment from the fact that the snout of the pig, the seat of the transpiration, is very liable to small vascular lesions which might

[1] *Berl. klin. Wchnschr.*, 1891, S. 505.

furnish the bacteria that developed on Brunner's plates. Nevertheless, even in the case of pathogenic organisms, which swarm in the blood, the sweat is usually free from them. This has been shown by Krikliwy[1] in the case of cats inoculated with anthrax whose sweat, in spite of the passage of numerous bacteria into the circulation, contained none.

Micro-organisms, then, after their entrance into the refractory animal, are not eliminated by any of the excretory channels which serve for the elimination of many of the soluble poisons. It was necessary therefore to seek some other process capable of affording an explanation of the disappearance of the micro-organisms which so often and by such varied means make their way into the interior of a resistant organism. For it is a well-established fact that in these cases the micro-organisms do disappear completely. This has been observed so often that it is unnecessary to offer any demonstration of the fact. Perhaps in the refractory organism the micro-organisms undergo the fate of the foreign bodies which penetrate, or which are introduced, into the circulation. It has long been known, thanks especially to the work of Hoffmann and Recklinghausen[2], and of Ponfick[3], that particles of carmine or vermilion when injected into the blood are deposited in several organs. They are found in the spleen, the lymphatic glands and the bone-marrow. A certain number of these foreign particles may even be fixed in the liver and kidneys, but, instead of passing into the bile and the urine, they remain lodged in the interstitial tissue of the organs. The observers just cited noted that the coloured granules do not remain long in either the blood or the lymph but will be found in the interior of the cellular elements. These granules persist for weeks without any appreciable modification, differing in this from the micro-organisms which, as a rule, after several days or even after a few hours, disappear from the refractory organism. This disappearance might be more justly compared to the resorption of corpuscular elements which
[50] results in a more or less complete atrophy. The facts concerning the resorption of pus, of extravasated blood, of the mucosa of the uterus in pregnancy, etc., have long been known, and it is among these that one should seek analogies with the disappearance of the micro-organisms. When bacteria of various species are injected into

[1] *Vratch* (in Russian), St Petersburg, 1896, Nos. 8, 12.
[2] *Centralbl. f. d. med. Wissensch.*, Berlin, 1867, No. 31.
[3] *Virchow's Archiv*, 1869, Bd. XLVIII, S. 1.

refractory or not very susceptible animals, we always observe a local reaction in the form of inflammation, accompanied by the appearance of white corpuscles. Gradually the organisms disappear from the point at which they are introduced; the exudation becomes sterile and ultimately is completely absorbed. Numerous researches, which will be set forth in the succeeding chapters, have, indeed, demonstrated the remarkable analogy that exists between the disappearance of the micro-organisms from the refractory animal and the resorption of corpuscular elements or of animal cells.

The analysis of the phenomena of this resorption will help us considerably in our study of immunity against micro-organisms. When in any part of the animal organism a collection of pus, an effusion of blood, or any other organic lesion is produced, these lesions are usually repaired after the lapse of a longer or shorter interval. In those cases where the cells retain their integrity, they are taken into the lymphatic vessels and then pass into the circulating blood. In the course of his researches on the transfusion of blood, Hayem[1] observed "that blood injected into the peritoneum is absorbed unaltered and passes with its anatomical elements into the general circulation." He was able to demonstrate "that the lymphatic channels play an important part in this absorption." Lesage of Alfort[2] confirmed this result. He found that in the dog "one hour after an abundant haemorrhage into the peritoneum, induced experimentally, the red corpuscles commenced to pass freely, without alteration and in very large numbers, into the thoracic duct." I have observed a similar resorption of the red blood corpuscles of the guinea-pig when injected into the peritoneal cavity of other individuals of the same species. The white corpuscles can also be taken up by the lymphatic vessels without being modified in any way. At the end of an inflammatory reaction of feeble intensity, set up in cold-blooded animals, especially in the tadpole, the direct passage of leucocytes from the exudation into the lymphatic system may be observed.

The examples I have just cited are, however, quite exceptional. [51] In the great majority of cases the cellular elements that are undergoing resorption are seized by the amoeboid cells and are taken into their substance. Even in the resorption of the red corpuscles, lying free in the peritoneal cavity of the same species of animal, a certain

[1] *Compt. rend. Acad. d. Sc.*, Paris, 1884, t. XCVIII, p. 749.
[2] *Compt. rend. Soc. de biol.*, Paris, 1900, p. 553.

number of the globules do not pass directly into the circulation but are first ingested by the amoeboid elements. This fact is insisted upon by Lesage. In inflammatory exudations the leucocytes also become the prey of their fellows. The ingested white corpuscles may be recognised for some time lying in the interior of other leucocytes; they are soon broken up, however, and finally disappear completely. When, instead of isolated cells such as leucocytes, we introduce fragments of tissues or of organs into any part of the organism, the same mode of resorption may always be observed. The introduced fragments are first surrounded and infiltrated by amoeboid cells and are then taken up into their interior.

The mode of absorption just described is very general. It applies to all kinds of cells and is observed in the absolutely normal organism, as well as in a large number of pathological conditions. For more than fifty years, the existence of cells which contain red blood corpuscles ("blutkörperchenhaltige Zellen" of German writers) has been recognised; they were met with in the spleen, the lymphatic glands and in many pathological products. For long we could not explain how the red corpuscles come to be inside other cells. Virchow[1] thought that they got there as the result of a mechanical pressure. Later histologists succeeded in determining the true nature of cells containing red blood corpuscles and in recognising that the leucocytes had really ingested the corpuscles. There has been much discussion, also, on the presence of leucocytes in the interior of large cells in exudations. It was thought that these were mother-cells which contained a new generation of small cells. Writers even described a fusion between the large cell and those found inside it; but Bizzozero[2] first recognised that the former was an amoeboid
[52] cell which had ingested pus corpuscles. Since this observation was made numerous cases have been described in which different cell elements have been found in the large cells. There could no longer be any hesitation in interpreting these cases as instances of ingestion by leucocytes or similar cells.

The changes that the ingested elements undergo within amoeboid cells may be compared with those that take place in intracellular digestion. If the modifications of the particles ingested by the *Amoebae* be studied side by side with those which take place in ingested

[1] *Virchow's Archiv*, 1852, Bd. IV, S. 536.

[2] "Handb. d. klin. Mikroskopie," 1887, S. 108; *Gaz. med. lombarda*, 1871 and 1872; *Wien. medic. Jahrbücher*, 1872, S. 160.

cells in the process of resorption, a striking analogy may be observed. To establish this satisfactorily it is essential to begin with a study of intracellular digestion properly so called, especially as in this phenomenon we have the fundamental basis of the whole of the theory developed in this work.

In our first two chapters we have already cited examples of this intracellular digestion in the Protozoa (*Amoebae,* Infusoria, etc.) and in the plasmodium stage of the Myxomycetes. In all these cases it goes on in the organism, in a distinctly acid medium, by the aid of ferments which could be demonstrated in the *Amoebae* and Myxomycetes, and which are analogous sometimes with trypsin, sometimes with pepsin.

In the lower Invertebrata we find the principal source of our knowledge of intracellular digestion in the digestive organs. This form of digestion is met with in Sponges, in the whole of the Coelenterates (Medusae, Siphonophora, Ctenophora, etc.), in the great majority of the Turbellaria (Planarians, Rhabdocoela), and in certain of the Mollusca (the lower Gasteropods). In the Invertebrata higher in the animal scale, intracellular digestion in the digestive organs becomes more and more rare, and sometimes it manifests itself only in the larval condition (*Phoronis*); ultimately it gives place permanently to digestion by juices secreted into the gastro-intestinal canal.

In his sketch of the comparative physiology of digestion, Krukenberg[1] sought to establish two types: protoplasmic or cellular digestion and secretory digestion. The former is effected, according to this observer, by a vital action independently of any production of soluble ferments. Secretory digestion alone, characteristic of the Vertebrates and of almost all the higher Invertebrates, is effected by means of these ferments (diastases or enzymes). Many observers, adopting this view, maintain that intracellular digestion presents [53] a purely vital phenomenon essentially different from that of chemical digestion due to juices containing soluble ferments secreted in the gastro-intestinal canal. That this theory is absolutely erroneous the succeeding pages of this work will furnish ample proof.

The Protozoa, from their small size, are unsuitable for researches on the essential phenomena of intracellular digestion. Amongst animals higher in the scale the Planarians lend themselves most readily to the observation of this process. These flat worms are very common in both fresh and sea water and are easily fed in captivity. They are

[1] "Grundzüge einer vergl. Physiologie der Verdauung," Heidelberg, 1882.

very voracious animals and, among other things, devour the blood of man or animals with avidity. One has merely to allow them to fast for a few days, and then to give them a drop of blood in order to see their digestive canal fill itself with this fluid (fig. 6). The white Planarian, *Dendrocoelum lacteum*, is well adapted for these researches. In a worm that has sucked blood from a Vertebrate, owing to its great transparency, the whole length of its intestine with its numerous ramifications may be seen. For some time this organ remains of a bright red colour, but gradually the tinge becomes brownish or faintly violet. These changes of colour recall those observed in effusions of blood in or under the human skin resulting from contusions. A microscopical examination of Planarians that have been fed with blood shows that the coloration of their digestive canal is due to red blood corpuscles in different stages of digestion. Immediately after the taking in of the blood by the Planarian all the red blood corpuscles are ingested by the epithelial cells of the intestine. Connected with the wall by slender

Fig. 6. Young Planarian some time after having sucked goose's blood.

[54] stalks, these elements appear as large amoeboid cells whose free end projecting into the lumen of the intestine sends out protoplasmic processes which seize the red blood corpuscles and convey them into the interior of the cell. This goes on very rapidly, and in a very short time all the red corpuscles are found within the epithelial cells. As a result of the increase in volume of these cellular elements the intestinal cavity is completely occluded.

[55] Once inside the cells of the intestine the red blood corpuscles exhibit changes which are readily followed under the microscope. It is better still to feed the Planarians with the blood of those lower Vertebrates whose red corpuscles are nucleated. In my researches I have used the blood of the goose. The red blood corpuscles of this bird, when ingested by the epithelial cells of the intestine of Planarians, are usually collected into compact groups (fig. 7), only a few remaining isolated. The majority of these red corpuscles soon lose their normal appearance and contour; they become

rounded and fused together, but the nucleus and the haemoglobin enable us to recognise them without any difficulty. Later

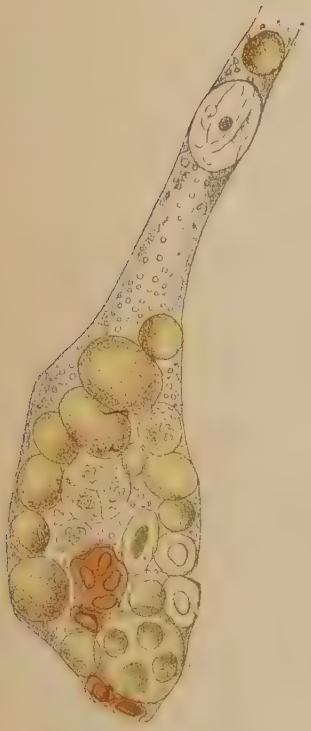

Fig. 7. Intestinal cell of a Planarian, filled with red blood corpuscles, undergoing digestion, of the goose.

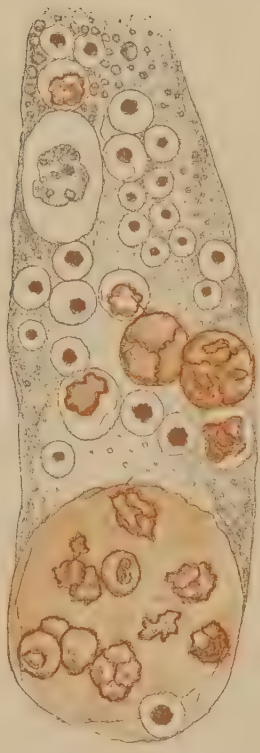

Fig. 8. Digestion of red blood corpuscles of the goose within an intestinal cell of a Planarian.

the red colouring matter begins to diffuse into the digestive vacuoles which form around the corpuscles. These corpuscles empty themselves, retaining their nuclei and capsules, which shrivel more and more. The nucleus also undergoes almost complete digestion, its membranous layer alone persisting (fig. 8). Even several days after the digestion of the blood has begun one can still find *debris* of perfectly recognisable red corpuscles, but the red colour has been replaced by a more or less pronounced brown tint. In the last stage of the digestive process, as the red corpuscles disappear, the

protoplasm of the intestinal cells becomes filled with round vacuoles, containing brown irregular concretions—excreta—which are expelled into the intestinal cavity.

This slow digestion of a substance usually so easily assimilable as blood takes place entirely within the epithelial cells of the intestine. Continuous microscopical observation demonstrates most clearly the complete absence of any extracellular digestion of the blood corpuscles in the intestinal content.

[56] When goose's blood mixed with blue litmus powder is given to Planarians, the coloured grains may be found some hours afterwards inside the epithelial cells of the intestine, but only a few of the blue litmus granules change colour, taking on a light violet tinge ; the great majority retain their blue coloration. It might be concluded from this that in Planarians intracellular digestion is effected in a neutral or nearly neutral medium. If, however, the preparations of intestinal cells gorged with goose's blood are treated with a 1 % solution of neutral red, we at once notice that the red corpuscles and the vacuoles which contain them are stained bright red, assuming a tint similar to that given with picrocarmine staining (fig. 9). This colour reaction indicates, according to our researches on neutral red, an acid reaction, more feeble, however, than that met with in *Paramaecium* and many other Protozoa.

Fig. 9. Portion of an intestinal cell of a Planarian, treated with 1% neutral red.

Macerations of Planarians in normal saline solution to which has been added a small quantity of the red corpuscles of the goose's blood exhibit *in vitro* a very distinct solvent action on these corpuscles, which become rounded and lose their haemoglobin, this latter diffusing into the surrounding fluid, and at the close of the experiment there remain simply the membranes and the nuclei of the corpuscles.

The study of these Planarians shows us, then, that the food of these animals undergoes exclusively intracellular digestion in a feebly acid medium and by means of a soluble ferment, and it furnishes us with proof that typical intracellular digestion is essentially a chemical process due to the intervention of enzymes. Now there can be no question, here,

of a protoplasmic action proper, but the branched digestive canal, so intimately associated with the parenchyma, cannot be completely isolated from the rest of the Planarian, and it is impossible to study *in vitro* its digestive action apart from other tissues. To attain this end we must turn to animals of larger size and those in which the digestive organs can be isolated more easily. In the Coelenterata intracellular digestion is general. Many of them are so transparent that they can be examined *in vivo*. It is easy to observe that the particles of food are seized by amoeboid processes of the entodermic cells and that they pass into the substance of these elements there to be digested. For the systematic study of the digestive phenomena, however, it is not sufficient merely to examine all that takes place in the living animal. Experiment *in vitro* is also necessary. For this [57] purpose the Actinians or sea-anemones offer us really excellent material. As these animals are very common in all our seas and are easily kept alive for long periods in aquaria, they have been used for various researches, among others for the study of the process of digestion.

The Actinians are easily fed in captivity; they devour morsels of flesh, of shrimps, of mollusca and other marine animals with avidity. The ingenious English observers Couch and G. H. Lewes[1] long ago demonstrated that morsels of food when introduced enclosed in perforated quills or wrapped in test paper or gutta percha silk and swallowed by the anemones were afterwards ejected surrounded by mucus but with no trace of digestion. Having failed in their search for digestive juices in the large gastric or coelenteric cavity of the Actinians, Lewes concluded that digestion in these animals is effected in a purely mechanical fashion. The greatly developed muscles of the Actinians were supposed to squeeze the food and extract its fluid which is then absorbed by the walls of the general cavity. It was not until very much later that the problem of digestion in the Actinians could be resolved in any accurate and definitive fashion. More than twenty years ago I demonstrated[2] that the digestion in these polyps is intracellular. In order that a clear conception of this phenomenon may be obtained it may be useful to recall in a few words the fundamental features of the organisation of Actinians. They are cylindrical bodies, sometimes as large as the fist, attached by their base to stones, shells, or other submarine objects, and furnished at their free extremity with one or more series of tentacles. In the middle

[1] G. H. Lewes, "Sea-side Studies," Edin. and London, 1858, p. 216.

[2] *Zool. Anz.*, Leipzig, 1880, Jahrg. III, S. 261, and 1882, Jahrg. V, S. 310.

of this extremity is an elongated opening, the mouth, which leads into a spacious sac, often spoken of as the stomach. It is, however, only a kind of oesophagus, through which the food passes into the large coelenteric cavity which is divided by septa into numerous compartments lined by the entodermic epithelium. These septa give origin to many very long and tortuous filaments, spoken of as mesenterial filaments from their resemblance, a purely superficial one, to the mesentery of higher animals (fig. 10). When the Actinian is hungry it protrudes its tentacles in order to seize marine animals, which it conducts to its mouth. The lips and the oesophagus are
[58] used to estimate the quality of the capture, and if it is found unsuitable the anemone rejects it, first surrounding it with a layer of

Fig. 10. Longitudinal section of an Actinian (after Hollard).

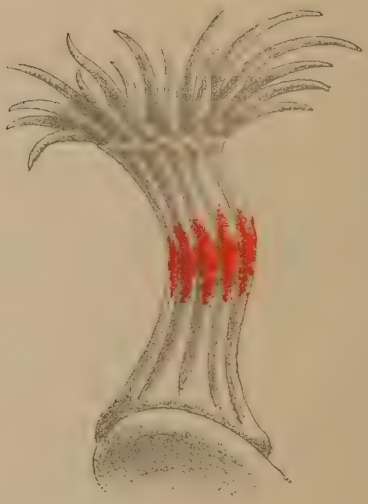

Fig. 11. An Actinian in which carmine after absorption has passed into the mesenterial filaments.

mucus. If however the food is found to be suitable, the Actinian retains it in its large cavity and throws around it a multitude of its mesenterial filaments. These penetrate it in all directions, and as their epithelial cells are capable of sending out amoeboid processes they seize and ingest the particles, which immediately enter the protoplasmic content. This work is done with such precision and nicety that the sea-anemone is able to extract the contents of a shrimp from the carapace, which latter alone it rejects.

The epithelium of the mesenterial filaments is therefore the organ of digestion in the Actinians. The nutritive parts of their prey pass into the amoeboid epithelial cells and there undergo a purely intracellular digestion. If we add to the shrimp-muscle or other food a little carmine or blue litmus powder, the mesenterial filaments ingest it also and become pigmented. After eating carmine they assume a very brilliant rose colour (fig. 11); blue litmus colours them [59] rose violet. This change of colour in the interior of the cells of the filaments indicates a decidedly acid reaction of their contents[1]. When one adds to the mesenterial filaments which are carrying on the process of digestion a drop of a 1 % solution of neutral red they assume various shades of red (fig. 12).

This intracellular digestion in the Actinians has been confirmed by several observers, amongst whom may be cited Chapeaux[2] and Bjeloussoff[3]. It has often been asserted, however, that, along with a digestion in the interior of the cells of the mesenterial filaments, there is, in the Actinians, a secretion in the coelenteric cavity of their body of fluids which digest nutritive matter by means of a soluble ferment. A ferment similar to trypsin has been extracted from Actinians by Léon Frédéricq and Krukenberg. But, in presence of contradictory assertions, it remained undecided whether, in the enzymatic digestion, this ferment does its work in the fluid of the coelenteric cavity or whether it represents the active factor in intracellular digestion.

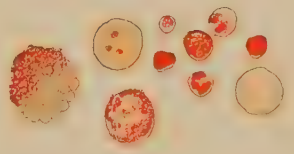

Fig. 12. Portion of mesenterial filament of an Actinian, stained with 1 % neutral red.

With the object of definitely elucidating a problem of such general importance, Mesnil, the superintendent of my laboratory, has been good enough to carry out a fresh series of experiments on the digestion of the Actinians and has studied this process not only in animals kept in captivity in aquaria but also in Actinians living under natural conditions in the sea[4].

As intracellular digestion is of interest to us specially in connection with the resorption of formed elements in the tissues and cavities of

[1] Metchnikoff, *Ann. de l'Inst. Pasteur*, Paris, 1893, t. VII, p. 348.

[2] *Bull. Acad. roy. de Belg.*, Brux., 1893, t. XXV, p. 262, and *Arch. de Zool. expér.*, Paris, 1893, 3me série, t. I, p. 139.

[3] "Etudes de physiologie sur les Actinies," Charkoff, 1895 (in Russian).

[4] *Ann. de l'Inst. Pasteur*, Paris, 1901, t. XV, p. 352.

animals, Mesnil directed his attention to the digestion of the red corpuscles of the blood. He made use of the red corpuscles of
[60] several species of Vertebrata, but he made a special study of the digestion of nucleated red blood corpuscles. These corpuscles are very delicate, and may even undergo a certain degree of maceration in ordinary sea water. In spite of this these red corpuscles are not digested in the coelenteric cavity of the Actinians but, once ingested by the entodermic cells of the mesenterial filaments, they are completely dissolved by the intracellular digestion. Mesnil also observed that fibrin is not digested except in the cells of the filaments. The facts cited by Chapeaux in favour of an extracellular digestion in the fluid of the coelenteric cavity in no way support his hypothesis, and reduce themselves, according to Mesnil, to a digestion by the diastase of blood itself fixed by the fibrin, after the bleeding, at the moment of the formation of the clot.

For a certain period the red corpuscles may be met with inside the cells of the mesenterial filaments. They are ingested in their normal state—oval red corpuscles with a nucleus. As several hours are required for the ingestion, it is evident that the fluid of the coelenteric cavity has been incapable of attacking the red corpuscles. In the protoplasm of the entodermic cells the red corpuscles become rounded, their walls become permeable, and the haemoglobin begins to diffuse from them. It passes first into the vacuoles of the digestive cells and is then, in part, ejected into the general body cavity. The haemoglobin is transformed into a green substance which reminds one of biliary pigment. The membranes and nuclei of the red corpuscles are also digested and ultimately disappear completely.

The digestive cells of the entoderm ingest not only blood corpuscles or fibrin, but also fragments of muscular fibre and particles of carmine and litmus. These latter, as already stated, indicate a marked acid reaction.

In the Actinians, then, the mesenterial filaments, or rather their entodermic portion, represent the real organ of intracellular digestion. There are indeed other regions of the entoderm which also carry on this function, but in an insignificant degree as compared with the mesenterial filaments which are capable, however, not only of ingesting and digesting solid substances, but also of absorbing solutions. Mesnil has demonstrated this by injecting soluble
[61] colouring matters, such as eosin, carminate of ammonia, etc., into Actinians. These solutions, although in great part absorbed by the

digestive cells of the mesenterial filaments, can, however, also be retained by other elements, amongst others, the cells of the ectoderm.

As the digestion of the food-particles goes on within the entodermic cells of the mesenterial filaments and as these organs can easily be isolated from the rest of the Actinian, Mesnil was able to study with great precision and care the phenomena of digestion outside the organism. With this object he prepared extracts of the filaments in sea-water and studied their action on various nutritive substances. He confirmed the discovery of a soluble ferment made by Léon Frédéricq and demonstrated that it is capable of digesting albuminoid substances (fibrin, coagulated albumen) in media which are neutral, slightly alkaline or weakly acid. In this respect the *actino-diastase* (the name given by Mesnil to the soluble ferment of the Actinians) approaches most nearly to papain. On the other hand, it is distinguished by its greater sensitiveness to an excess of acid and also by its more powerful action on coagulated albumen.

The actino-diastase acts vigorously at any temperature between 15° and 20° C., but the optimum temperature for its digestive action is between 36° and 45° C. Higher temperatures weaken the diastatic power, and heating to 55—60° C. inhibits it completely. Among the products of the digestion of albuminoids by actino-diastase, Mesnil, like his predecessors, found not only a notable quantity of peptone but also products of the disintegration of the albuminoid molecule, such as tyrosin and proteino-chromogen. Consequently actino-diastase resembles Mouton's amoebo-diastase in certain respects.

The nucleated red blood corpuscles of the lower Vertebrata are very convenient objects on which to observe the process of intracellular digestion within the cells of the mesenterial filaments. Mesnil has also studied them *in vitro* under the influence of actino-diastase. Under these conditions the phenomena of digestion recall very clearly those that have been observed within the digestive cells. The oval red corpuscles of the fowl and goose become spherical as a result of the solvent action on their membrane, and the haemoglobin diffuses into the fluid. The membranes and the nuclei of the corpuscles are, however, little altered and may be recognised under the microscope. The difference between this and digestion within the cells reduces itself to a more feeble digestive action of the aqueous extract. It is evident that the preparation of this extract is only capable of bringing into [62] prominence a certain proportion of the actino-diastase contained in the entodermic cells of the filaments.

Mesnil has fed the same Actinians with repeated doses of blood with a view to make out whether the cells, under these conditions, acquire any special aptitude for the production of the actino-diastase. Notwithstanding numerous attempts, he could never assure himself that this takes place ; the rapidity with which the red corpuscles were dissolved by the extract of the mesenterial filaments was the same whether this was prepared from Actinians that had been several times fed on blood or from those that had received none at all.

From what I have just described no doubt can exist that intracellular digestion is not a "protoplasmic" process essentially different from that which is brought about by the digestive juices secreted in the intestinal canal. In both cases we have a diastatic action, due to soluble ferments, produced by living elements. In intracellular digestion, however, the diastases carry on digestion in the interior of the cells, principally in the vacuoles, whilst in extracellular digestion this process goes on outside the cells, in the lumen of the gastro-intestinal canal.

It cannot be doubted that, in the animal scale, intracellular digestion represents an earlier and primitive condition for the solution of the food substances. This follows from the fact that it is widely distributed amongst the lowest animals, such as the Protozoa, Sponges, Coelenterata and Turbellaria. Intracellular digestion only gives way step by step to digestion by secreted juices. The higher Invertebrata furnish us with conclusive testimony on this point. Thus, among the gasteropod Mollusca, there are some which exhibit the two modes of digestion in the same animal. In *Phyllirhoë*, a beautiful mollusk, without a shell and quite transparent, which floats on the surface of the sea, the food can be seen passing into the cavity of the digestive canal, where it undergoes a preliminary digestion by secreted juices ; the result is a magma of small solid particles which are at once seized by the amoeboid epithelium of the coecal appendages, two on each side of the body. Intracellular digestion then completes the process and ends by dissolving the nutritive substances and reducing them to their final stage previous to absorption. On adding to the food some particles of carmine these may be found along with the digestible particles in the interior of the epithelial cells of the coeca.

[63] This example furnishes us with a real link between primitive intracellular digestion and the perfected and derivative extracellular digestion. In the same group of Gasteropods may be followed out

several stages of this evolution so that in the higher representatives of the group, such as the slugs and the snails, we meet with digestion carried on only by secreted juices in the gastro-intestinal contents. In these Mollusca a voluminous glandular organ, the liver, which is certainly derived from coecal appendices similar to those of *Phyllirhoë*, is now met with. Regarded from this point of view the liver is, as Claude Bernard has stated, an organ of second digestion. I think that a detailed study of the liver of the Mollusca, guided by this idea, will give results of considerable importance.

In the Vertebrata intracellular digestion in the gastro-intestinal canal almost disappears and is replaced by digestion carried on by means of ferments contained in secreted juices. We cannot, of course, offer to the reader anything like a complete account of this extracellular digestion in the higher animals. It is necessary, however, to draw attention to several aspects of this function which have been established, thanks to the progress made during recent years, in obtaining digestive juices and in the study of their action.

For the study of intracellular digestion the sea-anemone is the most suitable animal for our purpose; for that of extracellular digestion the dog. In this latter animal, an omnivorous flesh-eater, the food-substances are treated by digestive juices of great activity which contain a whole series of soluble ferments. The stomach secretes two of these: rennet and pepsin. The pancreas elaborates three: trypsin, amylase and saponase, which act on the three main groups of food-substances. To these the small intestine adds a special ferment, described by Pawloff[1] under the name of enterokynase. Every one recognises the proteolytic function of pepsin and trypsin and the analogies and differences between these two diastases. Nor need I dwell on amylase or on the ferment which saponifies fats. But enterokynase merits special attention in connection with the study of immunity. Pawloff entrusted to his pupil Chépowalnikoff the study of the digestive *rôle* of the intestinal juice concerning which, up to this, very little was known. It was known indeed that this juice contained weak saccharifying and inverting ferments, but it was [64] generally regarded as a secretion of little importance. Chépowalnikoff[2] has demonstrated that this view is absolutely erroneous. The intestinal juice fulfils the very important function of accelerating the

[1] Address delivered before the *Société des médecins russes* at St Petersburg. *Gaz. clin. de Botkine*, 1900.

[2] "Physiologie du suc intestinal," Saint-Pétersbourg, 1899 (Thesis, in Russian).

action of the three pancreatic ferments. The duodenal juice of the dog, especially, contains enterokynase. When this juice is mixed with a pancreatic juice that by itself actively digests fibrin and albumen, digestion takes place still more rapidly, the action being from three to four times as great. The part played by the intestinal juice becomes even more evident when it is mixed with a pancreatic juice that has little or almost no activity, as is the case of that from dogs that have recently been operated upon. Thus pancreatic juice, which has no action upon albumen, digests it promptly when a certain quantity of duodenal juice is added. When Chépowalnikoff took 500 c.c. of inactive pancreatic juice diluted with 500 c.c. of water or soda solution and added to it but a single drop of intestinal juice, the mixture exerted a manifest digestive action on coagulated albumen.

If, in place of pancreatic juice, we take the aqueous or glycerinated extract of the pancreas, which by itself exerts a very insignificant digestive action on albumen, and add to it intestinal juice, digestion takes place immediately. If it be admitted, as several physiologists maintain, that the inactivity of the pancreas is due to the fact that we have zymogen present in place of trypsin, one might conclude with Chépowalnikoff that "the intestinal juice possesses the power of transforming the zymogen into trypsin, and that this transformation takes place in a much more marked degree than in the presence of acids or the oxygen of the air" (p. 137).

The intestinal juice, from whatever region of the small intestine it be derived, exercises an undoubtedly favourable influence on the digestion of starch by the pancreatic juice, but this action is much more feeble than that on trypsin digestion. The action of the intestinal juice on the saponification of fats is even less marked. But here it is to the bile that the more important *rôle* is transferred. This fluid also augments the activity of the pancreatic juice, but in a manner different from the intestinal juice, for it acts especially by accelerating the digestion of fatty substances.

[65] The action on the pancreatic digestion is not in any way interfered with when the bile is heated to boiling point. On the other hand the intestinal juice, under these conditions, completely loses its accelerating *rôle*. It follows from this, as has been formulated by Pawloff, that, in the intestinal juice, the existence of a soluble ferment which is destroyed by heat must be admitted; to this ferment he proposes to give the name of enterokynase. Without exercising

a digestive power on any of the alimentary substances, it may act as a ferment of the pancreatic ferments.

Delezenne, at the Pasteur Institute, has repeated Chépowalnikoff's experiments. He has confirmed the accuracy of his results and has added new data of great importance, not only as regards the physiology of digestion but also in relation to the study of immunity. Enterokynase appears from Delezenne's experiments to be a true ferment; carried down by the same precipitants (collodion, phosphate of lime, alcohol) which enable us to obtain the greater number of the known ferments; it is sensitive to high temperatures, and even that of 65° C. is sufficient to do away with the greater part of its activity. Yet another property of enterokynase, which it possesses in common with the soluble ferments and which has for us a very special interest, is the facility with which it attaches itself to fibrin. By means of flakes of this substance we can at any time remove from a fluid the whole of the enterokynase contained therein. This fixative property is very important in connection with the part which enterokynase plays in digestion. The fibrin to which it has become attached absorbs trypsin with great avidity. If we introduce flakes of fibrin impregnated with enterokynase along with other flakes which have not been in contact with this ferment into a solution of trypsin, the former are digested with great rapidity, whilst the latter do not undergo any change. The fibrin that has fixed enterokynase is capable of clearing a fluid of its trypsin. On the other hand, that which has not been acted upon by the intestinal juice leaves it there almost unaltered.

It is of the utmost importance that we should inform ourselves as to the origin of the enterokynase of the intestinal fluid. This fluid, when obtained from a fistulous opening, for example, contains mucus and a considerable amount of *débris* of various kinds of cells. What are the elements which furnish such a remarkable ferment? Dele- [66]
zenne has obtained a very precise answer to this question. The enterokynase is not contained in the mucus and is not secreted by the intestinal glands; it comes from the lymphoid organs.

If the small intestine of a fasting dog be washed carefully with water all the pre-existing enterokynase is removed from it. The Peyer's patches are then removed and treated with chloroform water. The other parts of the small intestine are similarly treated. This fluid dissolves the enterokynase, as it does the other soluble ferments. We find that the Peyer's patches furnish enterokynase, but that the rest of the intestine, including Lieberkühn's glands, give none.

We know that the Peyer's patches are lymphoid organs in which are a large number of amoeboid mononucleated cells, and that these elements are even capable of ingesting foreign bodies and of submitting them to intracellular digestion. It is therefore not at all astonishing that Delezenne should have succeeded in finding enterokynase in the mesenteric glands of several Mammals (dog, pig, rabbit). These glands, when treated by the method just mentioned, yield a substance which assists the action of trypsin just as does the intestinal juice. Having reached this point, Delezenne asked himself whether the mononucleated white corpuscles, so closely allied to the mononucleated cells of the lymphoid organs, may not also contain enterokynase. With the object of settling this point he collected exudates that were rich in mononucleated leucocytes ; in these also he found this same soluble ferment. Moreover, the leucocytic layer of the blood showed itself equally capable of increasing, very energetically, the action of trypsin.

The results of the old experiments carried out by Schiff and by Herzen on the adjuvant *rôle* of the extract of the spleen in pancreatic digestion, must without doubt be ranged alongside those we have just indicated. In fact the mononucleated cells of the spleen, like those of Peyer's patches and of the mesenteric glands, contain a substance which acts like enterokynase. Delezenne has given us a definite demonstration of its presence and action.

In intracellular digestion it is the chemical side which has been most difficult of demonstration. The purely physiological functioning, the sensitiveness of the digestive cells and the amoeboid movements
[67] of their protoplasmic processes are, on the other hand, so manifest that it has even been suggested that intracellular digestion should be looked upon as a protoplasmic phenomenon purely vital in character.

In extracellular digestion through the agency of secreted juices we have a very different condition. Here the chemical side is the striking feature, the physiological factor being veiled more or less completely. Nevertheless, thanks to recent advances and above all to the labours of Pawloff's disciples in St Petersburg, this problem has been elucidated in a very remarkable fashion.

The secretion of digestive fluids follows definite laws, the most potent factor being the reflex action of the nervous system. To use the expression of Pawloff, the study of the process of salivary secretion has revealed a real psychology of these organs. You may fill the mouth of a dog with small polished pebbles or with snow ; you may pour into it very cold water—the saliva will not flow. But merely

allow the animal to see sand in the distance—the glands at once begin to secrete fluid saliva. Tempt the dog with flesh—and immediately a thick saliva appears ; show him dry bread—saliva is secreted in abundance, even if the dog has no great desire to eat.

The same phenomena may be observed in the stomach. Mechanical stimulation by inert bodies, such as stones, provokes no secretion ; but the suggestion of a meal or the sight of food is sufficient to call forth a large quantity of gastric juice. The quantity and quality of the gastric juice are regulated by the quantity and quality of the food. Bread given to a dog provokes the secretion of a gastric juice endowed with the greatest digestive power. That which flows after the ingestion of milk contains only one-fourth as much pepsin.

In spite of these differences in the gastric secretion in relation to food, Pawloff and his pupils have never been able to assure themselves that there was any prolonged and chronic adaptation of the gastric function. They were struck by the uniformity of the digestive power of a great number of their dogs. Samoïloff[1] had under observation three dogs placed on different diets. In spite of the very long periods during which these diets were given, the gastric juice, in all the dogs, presented the same properties and manifested no appreciable difference. This result harmonises with that indicated above as obtained in the Actinians fed with blood by Mesnil. In spite of repeated feedings on blood from the same species of animal, the extract from the mesenterial filaments was in no way different from [68] that of the fasting Actinians used for control.

The pancreatic secretion is, in many respects, a more perfect type. We have here to do with the principal agent in the digestive function, without which the organism could not continue to exist. The advances made in surgery have enabled us to remove the stomach, first in the dog and then in man, and there are already several persons[2] from whom the stomach has been removed and who, in spite of this operation, have continued to live. A portion of the small intestine may also be removed, but, in order that life may not be endangered, a considerable portion of it must be left intact. It is evident then that the pancreatic digestion is an admirably organised function both in animals and in man. One of the main regulators of this process of digestion consists in the great sensitiveness of the intestinal mucous membrane. Just as the organs of the buccal cavity possess in the

[1] *Arch. d. sc. biol.*, St.-Pétersb., 1893, t. II, p. 698.
[2] Cf. *Bull. Acad. de méd.*, Paris, 1901, p. 17.

specific sense of taste an excellent means of discrimination in the choice of foods, so the mucous membrane of the small intestine is endowed with a special sensitiveness, comparable to the chemiotaxis of unicellular organisms and of the cells of more highly developed organisms. Hirsch and Mehring have satisfied themselves that the passage of the contents of the stomach through the pyloric orifice depends on a reflex mechanism which proceeds from the upper reaches of the small intestine. To the researches of the school of Pawloff, however, we owe what light has been thrown on this question. The duodenal mucous membrane is endowed with a well-developed chemiotaxis for acid substances. The passage of the acid content of the stomach into the duodenum determines this chemiotaxis and brings about a secretion of alkaline juice which neutralises the acid. This contest between acid and alkali forcibly calls to our mind the analogous phenomena in those plants that defend themselves against the alkaline secretions of parasites by the production of an acid (see Chapter II). As in these lower organisms, this battle of the chemical secretions is regulated by the action of living and sensitive parts.

When the acidity of the mass which passes through the pylorus is too marked, the reflex contraction starting from the duodenal mucosa arrests its passage. Then takes place a neutralisation of the acid, thanks to the alkaline secretion, and the pylorus is again allowed to open. This mechanism thus regulates the passage of the contents of the stomach into the duodenum, the passage taking place in instalments.

[69] The sensitive intestinal mucous membrane can estimate not only the degree of acidity, but also the other chemical characters of the aliments which pass into the duodenum. This chemiotaxis is, as it were, the starting point of the reflex action which excites the pancreatic secretion with its contained three ferments. The passage of bread through the pylorus excites the secretion of a juice very rich in amylase and very poor in saponase. The passage of milk into the duodenum brings forth, on the other hand, a juice very much richer in saponase but poorer in amylase and in trypsin. Flesh-meat provokes the secretion of a pancreatic juice which is less rich in amylase than the juice poured on bread, but richer in saponase. Fat causes the secretion of a juice still richer in saponase than is the juice poured out in the presence of bread or milk. These facts now carefully established—especially by Walter[1]—demonstrate that the

[1] *Arch. d. sc. biol.*, St.-Pétersb., 1899, t. VII, p. 1.

pancreatic function is carefully regulated as regards its adaptation to the characters of the food substances on which it is to act. Such adaptation may even become permanent.

Whilst, as already stated, the stomach, under the influence of a fixed diet, is incapable of effecting any lasting modification in the composition of its secreted juice, the pancreas may reach this degree of perfection. When a dog is fed for several weeks on bread or on milk and is then placed on flesh diet its pancreatic juice is found to become progressively richer in trypsin. Whilst this augmentation of the proteolytic power is being brought about, the juice becomes poorer and poorer in amylase. Wassilieff[1] has carried out a large number of experiments on this point and has demonstrated a very remarkable adaptation of the pancreatic juice to the wants of nutrition, an adaptation that may become permanent. A dog which has been accustomed to digest bread and milk adapts itself to this nourishment: its pancreatic juice contains less and less trypsin, but, on the other hand, becomes richer in amylase. Pawloff observed that in dogs great variations in the composition of the pancreatic juice are often present; this he attributes to the diet to which these animals had been previously subjected.

Not only does the quality of the digestive juices accommodate itself to the wants of digestion; their quantity also undergoes variations according to the part that these juices have to play. Thus, Pawloff has observed that his dogs secreted a saliva which was very fluid and very abundant when he gave them acids, bitter substances or other sub- [70] stances they did not like. On the other hand, the presence of food in the mouth, or even the sight of it, excited the secretion of a thick saliva containing a large quantity of mucin. In the first case the part played by the saliva was that of diluting the injurious substances as much as possible, in the second that of facilitating the deglutition of the food.

In general the organism manifests a tendency to produce more digestive ferments than it actually needs for digestion. It is for this reason probably that they are often found outside the digestive canal. Among these ferments pepsin and amylase, especially, have been definitely proved to be present in the urine of man and of some mammals, notably the dog. The data as to rennet and trypsin are not so well established. But, as several of these ferments, such as amylase and trypsin, may be derived from several sources in the organism, their elimination by the urine is less important for the thesis I have just formulated than is that of pepsin.

[1] *Arch. d. sc. biol.*, St.-Pétersb., 1893, t. II, p. 219.

Pepsin was found in the urine by Brücke exactly forty years ago. It is more frequently found in the morning urine, but is absent from that passed immediately after the principal meal. Leo and Senator[1] found only traces of pepsin during the prolonged fast of the Italian Cetti ; but the day he broke his fast they were able to demonstrate the presence of a considerable quantity of this ferment in his urine.

Delezenne and Froin, with the object of seeking the source of the urinary pepsin, extirpated the stomach of a dog. After the animal had recovered, they fed it well and examined its urine at different periods of the day. By the methods which had shown the presence of pepsin in all the normal dogs taken as controls they could never discover the faintest trace of this diastase in the urine of the dog that had been operated upon. On the other hand, the urine of a dog whose stomach had simply been isolated, contained very much the same quantity of pepsin as that of normal dogs. This experiment proved among other things that the pepsin, before it could be eliminated by the kidneys, must have been re-absorbed by the wall of the stomach.
[71] From these data, combined, it must therefore be admitted that the pepsin found in the blood and which passes thence into the urine can only be of gastric origin. As it serves no useful purpose in the organism we must conclude that a portion of the pepsin, secreted by the stomach and not used for digestion, has been rejected as superfluous.

The study of the digestive function of animals gives us information on a large number of points of the highest importance for the comprehension of immunity. Intracellular digestion, a function so widely distributed in the lower animals, is very intimately connected with the phenomena which are observed when micro-organisms are destroyed in the animal organism. Extracellular digestion furnishes us with information concerning many of the features of progressive adaptation, similar to those which are observed in connection with acquired immunity.

When we examine the phenomena of intracellular digestion and those of secretory digestion as a whole, we see that, in both, the chemical processes are subjected to the influence of the living parts of the organism. In the lower animals, it is the protoplasm of the amoeboid cells which regulates the chemical processes in digestion ; in the higher animals, this *rôle* is taken by a very complicated apparatus, in which the nervous system plays a predominant part.

[1] *Virchow's Archiv*, 1893, Suppl. to Bd. CXXXI, S. 142. The question of urinary ferments is summarised in Neubauer u. Vogel's "Analyse des Harns," Wiesbaden, 10te Aufl., 1898, S. 599.

CHAPTER IV [72]

RESORPTION OF THE FORMED ELEMENTS

Digestion in the tissues.—Resorption of cells in the Invertebrata.—Resorption of red corpuscles by the phagocytes of the Vertebrata.—Phagocytes.—Various categories of these cells.—Macrophages and microphages.—Part played by macrophages in the resorption of the formed elements.—Digestive property of the macrophagic organs.—Solution of the red blood corpuscles by the blood serums.—The two substances which operate in haemolysis. Macrocytase and fixative.—Analogy of the latter with enterokynase.—Escape of the macrocytase during phagolysis. Suppression of phagolysis. Resorption of the spermatozoa.—Presence of fixatives in plasmas.—Origin of fixatives.

It is usually understood that nutritive substances must necessarily be subjected to the influence of the digestive juices in the gastro-intestinal canal before they can be utilised for the nutrition of the organism. This is a very old idea. It was based on a well-known experiment by Schiff who injected several animals intravenously with solutions of cane sugar and egg albumen and others with the same substances after they had been artificially digested. In the first case the food substances passed into the urine, in the second they only appeared there when injected in large quantities.

At the recent International Congress of Medicine held in Paris in 1900, the question of extra-buccal nutrition was much discussed[1]. It has been accepted that fats, when injected into the subcutaneous tissues, are, at least in part, absorbed by the organism, but that carbo-hydrates and albuminoids are never absorbed. This is perhaps true from the point of view of clinical medicine. But, in principle, it must be admitted that food substances of very diverse natures, when introduced into the organism by channels other than the gastro- [73] intestinal canal, still undergo profound changes.

[1] *Compt. rend. du XIII^e Congrès internat. de Méd.*, Paris, 1901. Leube, "Ueber extrabuccale Ernährung," in "Deutsche Klinik am Eingange d. XX. Jahrhunderts," Wien u. Leipzig, 1901, I, S. 64.

When we inject milk, blood serum, or white of egg, that is to say, materials very rich in albuminoid substances, under the skin or into the peritoneal cavity of laboratory animals, we find that after a time they disappear. At the same time they give rise to modifications of the organism which indicate that these injected substances have there undergone profound changes.

After injecting eel's serum into rabbits, Th. Tchistovitch[1] found a substance in the blood of the injected animals which gave a precipitate with eel's serum. Shortly afterwards Bordet[2] observed that the blood of animals into which he had injected cow's milk acquired a new property: it gave a precipitate with this milk, a condition never observed in the serum of untreated animals.

The injection of white of egg into rabbits, carried out by Myers[3] and Uhlenhuth[4], brought about the same changes in the blood serum. The researches of the latter of these two observers have for our present purpose a special interest. He demonstrated first that the injection of white of egg into the peritoneal cavity of rabbits was followed by the appearance in the blood serum of these animals of a substance which precipitates egg albumen *in vitro*. Uhlenhuth then obtained this same acquired property of the blood in rabbits which had been made to swallow a considerable quantity of the white of hens' eggs. Twenty-four days after the commencement of this regimen the serum of the rabbits precipitated white of egg in the test-tube. This example affords a marked analogy between the results of digestion in the alimentary canal and those of resorption into the tissues. Uhlenhuth points out, indeed, that his rabbits which received the injections of white of egg into the peritoneal cavity flourished under this treatment.

A certain number of similar examples are now recognised. They all indicate that various nutritive substances, when introduced into the peritoneal cavity or under the skin of animals, are retained there for a longer or shorter time and are subjected to certain modifying influences on the part of the organism. The proof that these
[74] substances are not eliminated intact by the kidneys has been furnished by a large number of experiments. Recently Lindemann[5] and Néfédieff[6], working in my laboratory, have established the fact

[1] *Ann. de l'Inst. Pasteur*, Paris, 1899, t. XIII, p. 406.
[2] *Ann. de l'Inst. Pasteur*, Paris, 1899, t. XIII, p. 225.
[3] *Centralbl. f. Bakteriol. u. Parasitenk.*, Jena, 1900, I^te^ Abt., Bd. XXVIII, S. 237.
[4] *Deutsche med. Wchnschr.*, Leipzig, 1900, S. 734.
[5] *Ann. de l'Inst. Pasteur*, Paris, 1900, t. XIV, p. 49.
[6] *Ann. de l'Inst. Pasteur*, Paris, 1901, t. XV, p. 17.

that normal blood serum, when injected under the skin of animals, does not provoke albuminuria at all, or at least produces it in a very insignificant and transitory degree.

The mechanism by which the organism modifies these nutritive substances, introduced by a channel other than the digestive canal, is not as yet sufficiently known; and is therefore not easy to define. But we know, very definitely, that each injection of serum, whether of white of egg, milk or fatty matter, is followed by a rather considerable aseptic inflammation at the point at which these substances are introduced. We might conclude from this that the organism digests the food substances outside the gastro-intestinal canal, by means of an inflammatory reaction. In order to determine more exactly the phenomena that appear under these conditions, it may be useful to consider first, not the fluid substances but the solid elements that are introduced into the tissues and cavities.

Let us begin with the lower animals in which the anatomical organisation and all the functions are of a much more simple character than they are in the Vertebrata. In my *Comparative Pathology of Inflammation* (Lecture IV) I have directed some attention to the digestion of the Sponges.

The nutritive substances—small organisms—whether they may have entered by the small openings, so numerous on the surface of Sponges, or have been introduced through a rent in the body wall, undergo the same fate. They are seized by vibratile or amoeboid cells which ingest the food and digest it by an intracellular digestion. These two kinds of cells, which come under the category of *Phagocytes*, have a great resemblance to one another, and we may say that digestion and resorption are two very closely related phenomena.

When we examine somewhat higher Invertebrata, such as the Medusae or certain other Coelenterates, we can still trace a close analogy between the true digestion of the food that goes on within the epithelial cells of the entoderm and the resorption of certain foreign bodies which make their way by an extra-buccal channel into the intermediary tissue. Here these bodies are surrounded by amoeboid cells which fulfil their function as phagocytes by ingesting [75] and digesting the substances that have come from outside.

It is, here, unnecessary to go over the whole gamut of the perfecting of the organisation of the Invertebrata, in its relation to the resorption of foreign bodies, especially as it has already been treated in my Lectures on Inflammation. Let us choose

merely some of the more common and better-known representatives of the Invertebrata and dwell for a few moments on the phenomena manifested in their organism, into the midst of which have been introduced a few nucleated red blood corpuscles[1].

If a small drop of defibrinated blood from a goose be injected beneath the skin of a snail and another under the skin of a cockchafer larva, the red corpuscles are disseminated in the blood fluid which, of itself, is incapable of modifying them, but at the end of a few hours the leucocytes of the two invertebrates that we have chosen for the experiment will have ingested a certain number of the injected red blood corpuscles. The next day red blood corpuscles are still to be found intact in the blood plasma, but the great majority have been devoured by the leucocytes (Fig. 13). Inside these cells the red corpuscles undergo constant and marked changes. In the snail they become round and their walls permeable. In the vacuoles that are produced around the ingested red corpuscles dissolved haemoglobin is found (Fig. 14); a portion of this colouring matter passes into the nucleus of the red corpuscles, so that it also has undergone a profound change (Fig. 14). Many of the nuclei become emptied, only the peripheral layer remaining. This layer and the membrane of the red corpuscle are the parts that resist the action of the leucocytes longest and they are found for some time after their ingestion. The white corpuscles of the snail, having devoured one or more red corpuscles, may themselves become the prey of their fellows.

In the "ver blanc" (French popular name for the larva of the cockchafer) the phenomena of resorption of the red corpuscles of the goose resemble those just described. The blood plasma leaves intact the red corpuscles which undergo no change until they have been ingested by the leucocytes. The haemoglobin diffuses into the leucocyte, whilst the nucleus and the membrane persist for a very
[76] considerable period (Fig. 15), though they lose their normal aspect, shrivel, and become transformed into an irregular mass of brown
[77] pigment which may remain in the substance of the leucocyte (Fig. 15, *p*) for weeks.

Having once injected goose's blood into snails and "vers blancs," if we repeat the injection several times, the phenomena observed are

[1] The resorption of the red blood corpuscles by the phagocytes of larvae of starfish (*Bipinnaria*) and of *Phyllirhoë* has been described in my paper on intracellular digestion in the Invertebrates in *Arb. a. d. Zool. Inst. d. Univ. Wien*, 1883, Bd. v, Hft. 2, S. 141.

invariably the same. The red corpuscles are unacted upon by the plasma and undergo the same changes within the leucocytes. These changes are in fact comparable to those described in the preceding

Fig. 13. Leucocytes of a cockchafer larva containing red blood corpuscles of a goose.

Fig. 14. Red blood corpuscles of a goose, free, and ingested by leucocytes of a snail (*Helix pomatia*), 24 hours after their injection.

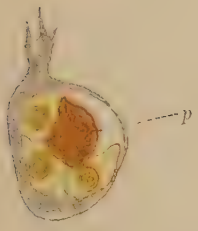

Fig. 15. Leucocyte of a cockchafer larva, 7 days after last injection of goose's blood.

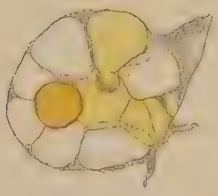

Fig. 16. Leucocyte from peritoneal cavity of a gold-fish after ingesting red blood corpuscles of a guinea-pig.

chapter in discussing the intracellular digestion of the red corpuscles by the intestinal cells of the Planarians. In both cases the red corpuscles are seized by amoeboid cells and subjected to the influence of

their contents. In the intestinal phagocytes of the Planarian, as in the phagocytes of the blood (leucocytes) of the snail and "ver blanc," the haemoglobin diffuses through the wall of the red corpuscle, whose most resistant parts are the nucleus and the membrane. These resistant residual fragments, impregnated with haemoglobin, become brown in the Planarian, in the "ver blanc," and also, but in a less degree, in the snail. The most appreciable difference consists in the formation of excretory vacuoles, containing concretions, in the Planarian, and the absence of these vacuoles in the blood phagocytes of the other Invertebrata. We have, however, less right to attribute a fundamental importance to this difference, in that the phenomena in the Actinians, which ingest the red blood corpuscles by the amoeboid cells of their entoderm, are in all respects (with the exception of the presence of these special excretory vacuoles) comparable to the phenomena observed in the Planarians. From the fact that in these two examples we have to do with a true intracellular digestion, it must be admitted that the modifications of the red blood corpuscles within the phagocytes of the blood in the snail and in the larva of the cockchafer, must also be placed in the same category of phenomena.

In order to make a more thorough study of this intracellular digestion in the phagocytes of the blood, we must direct our attention to larger and more highly organised animals than the snail and the "ver blanc." Let us take, first, an example among the inferior cold-blooded Vertebrata. The red blood corpuscles of a few drops (0·25 c.c.) of the blood of a guinea-pig injected into the peritoneal cavity of a gold-fish (*Cyprinus auratus*) are not appreciably changed by the peritoneal fluid itself; but the numerous leucocytes that are found in
[78] the peritoneal fluid seize them and ingest them, just as do the phagocytes of the blood of Invertebrata, or the intestinal phagocytes in the Planarians and Actinians in the case of the red blood corpuscles of the goose. Each leucocyte of the *Cyprinus* ingests several red blood corpuscles and subjects them to intracellular digestion. The stroma of the red corpuscles becomes permeable; the haemoglobin diffuses into the nutritive vacuoles and at the end of a shorter or longer period the whole is dissolved and decolorised (Fig. 16). Here no brown pigment is produced and the red corpuscles are completely digested, leaving no "remains"; in this respect differing from the process in the Invertebrata mentioned.

This result depends, probably, partly upon the more feeble resistance offered by the non-nucleated red corpuscles of Mammals, and

partly upon the more active digestive power of the leucocytes of Fishes.

As the result of several injections of guinea-pig's blood into the peritoneal cavity of *Cyprinus*, the peritoneal fluid acquires new properties[1]. If, a fortnight after the first injection, a little of the peritoneal exudation in the gold-fish be withdrawn, it is found that a drop of the serum which floats on the surface produces, almost immediately, well-marked agglutination of the red corpuscles of the guinea-pig, this being soon followed by the rapid solution of these red blood corpuscles in the fluid. This new property, which does not exist in the untreated fish, also makes its appearance in the blood serum of *Cyprini* treated with guinea-pig's blood. The experiment is very successful at a temperature of 18°—19° C.

As the solution or lysis of the red blood corpuscles in the serum is exactly like that which takes place within the leucocytes of *Cyprinus*, we are justified in assuming that, in both cases, it is produced by the same substance. And, since the solvent or haemolytic power of the serum is only acquired as the result of the intracellular digestion of the red blood corpuscles by the leucocytes, it is probable that the solvent substance represents the intracellular ferment derived from the leucocytes.

The subject we have just broached is of fundamental importance in connection with the study of resorption and of the phenomena of immunity dependent upon it. It is necessary, therefore, that we should go more fully into its analysis. With this object we must first review the processes that go on during resorption in the higher animals and continue our examination of the changes that injected [79] or extravasated blood undergoes in various positions of the organism.

This study is rendered comparatively easy for us by the numerous researches that have been carried out by pathological anatomists for the purpose of ascertaining the fate of effusions or extravasations of blood so frequently met with in disease. It has long been known that in subcutaneous, cerebral and other haemorrhages, or in hepatised lungs, there are found in the escaped blood a great number of cells containing red corpuscles. As was mentioned in the preceding chapter, these cells were evidently amoeboid cells that had ingested red blood corpuscles. To Langhans[2] especially we owe a detailed study of the

[1] I have only been able to discover the haemolytic property of the serums of *Cyprinus* after the third injection of guinea-pig's blood.

[2] *Virchow's Archiv*, 1870, Bd. XLIX, S. 66.

phenomena that follow extravasation of blood produced artificially in the subcutaneous tissue of the pigeon, rabbit and guinea-pig. In all these animals the haemorrhage is early followed by exudative inflammation, during which the leucocytes come up in great numbers and ingest the red blood corpuscles which are modified in the interior of the leucocytes. There is a formation or deposition of pigment and finally all traces of the red corpuscles disappear. In Mammals the pigment is brown or brownish, just as it is in the Planarians and in the "ver blanc"; in the pigeon it is green and resembles that found in the Actinians. In short there is a great analogy between the resorption of red corpuscles and the true intracellular digestion of the red blood corpuscles that goes on in the intestinal cells of the Invertebrata.

But what is the nature of these amoeboid elements that intervene in the resorption of the extravasated blood? At the period when Langhans carried out his investigation, we were unable to differentiate the cells at all satisfactorily. It is only since the publication of Ehrlich's classic researches on the white corpuscles that we have been able to bring more order into this question. Thanks to the use of various aniline stains, Ehrlich was able to arrange the leucocytes found in the Vertebrata into several definite groups.

The question has already been touched upon in our eighth lecture on inflammation; it is therefore unnecessary to treat it here at length. We must, however, before entering on the analysis of the essential phenomena in the resorption of cells, as we now understand them, give a rapid survey of the different varieties of amoeboid cells that are found in the Vertebrata.

[80] Beside mobile amoeboid cells, represented by several forms of white corpuscles, we must distinguish fixed amoeboid cells. These are permanently fixed in certain situations in the body; this, however, in no way prevents them from throwing out amoeboid processes in various directions and seizing foreign bodies or certain elements of the same organism. The nerve cells, the large cells of the splenic pulp and of the lymphatic glands, certain endothelial cells, the cells of the neuroglia, and perhaps some connective tissue cells, belong to the category of fixed amoeboid cells. All these elements, under certain conditions, are able to ingest solid bodies; consequently, they act as phagocytes. With the exception of the cells of the nerve centres, all these fixed phagocytes are of mesoblastic origin. It has been much discussed whether certain processes of the nerve cells may

not really serve to seize foreign bodies and carry them into the cell contents. It appears to us that sometimes they undoubtedly do fulfil this function. For example, it is only by means of such amoeboid movements that leprosy bacilli can be introduced into the interior of ganglion cells and cells of the spinal cord[1]. We must not dwell on this question, as the phagocytic property of the nerve elements plays no part in the resorption of cells. On the other hand, the neuroglia cells contribute largely to this process and their phagocytic function is now admitted by many observers[2].

For long the large "dust" cells of the respiratory channels were looked upon as being epithelial cells which were capable of ingesting carbon particles, micro-organisms and other foreign bodies. The researches of N. Tchistovitch, carried out in my laboratory more than twelve years ago, made it evident that these elements are nothing more than white corpuscles that have immigrated into the alveoli and bronchi.

It is probable that the same is the case as regards the stellate cells of the liver, known as Kupffer's cells. First described by Kupffer as cells of a nervous type, having long processes, they were later recognised by several observers as belonging to the endothelial [81] tissue of the blood vessels of the liver. Kupffer[3] himself has accepted this view and in his recently published monograph on these stellate cells, he describes them as endothelial cells that have retained their independence. Some researches on the resorption of blood, of which I shall speak shortly, have led me to think that these cells are nothing but white corpuscles that have been arrested in the hepatic capillaries. I have asked Mesnil, head of my laboratory, to study this question for me. His investigation is not yet concluded, but the demonstration already made that the livers of guinea-pig embryos and new-born rabbits do not possess any Kupffer's cells is an argument in favour of my hypothesis.

Certain white corpuscles have undoubtedly been often mistaken for epithelial or connective tissue cells. We must not conclude from this, however, that these elements are never capable of sending out amoeboid processes and of ingesting foreign bodies. It would, however, be useful to collect new and incontestable proofs of the

[1] Soudakewitch, *Ziegler's Beitr. z. path. Anat.*, Jena, 1888, Bd. II, S. 129, and Babes, "Untersuchungen über den Leprabacillus," Berlin, 1898, S. 58.

[2] Marinesco, *Compt. rend. Soc. de Biol.*, Paris, 1896, p. 726.

[3] *Arch. f. mikr. Anat.*, Bonn, 1899, Bd. LIV, S. 254.

accuracy of this thesis. In spite of this uncertainty, it may be accepted as fully demonstrated, that certain fixed amoeboid cells, such as the large elements of the splenic pulp, of the lymphatic glands, and of the omentum, play an important part in the resorption of cells. It is there that elements filled with red corpuscles and white corpuscles in process of being destroyed are so often found.

Just as certain fixed cells do not function as true phagocytes, so also in some leucocytes this function is undoubtedly absent. The suggestion has been made several times that any cell element, provided it be young, is capable of ingesting foreign bodies. The examination of white corpuscles proves exactly the contrary. The smaller white corpuscles found in fairly large numbers in the blood and the lymph, and which are commonly known as *lymphocytes* or *small lymphocytes*, are simply leucocytes with very little protoplasm which in this state never fulfil phagocytic functions. It is only when it becomes older, when its nucleus, single and rich in chromatin, becomes surrounded by an ample layer of protoplasm, that the lymphocyte becomes capable of ingesting and resorbing foreign bodies. Several
[82] authors, with Ehrlich at their head, still assign to these larger cells the same name—lymphocytes. Others, however, give them the name of large mononuclear cells. Confusion is thus possible, especially as Ehrlich includes under the same term the large mononucleated leucocyte, a very rare form of cell in human blood, which is distinguished by the greater staining capacity of its nucleus. To avoid this inconvenience I propose to designate the large lymphocytes by the name of blood macrophages and lymph macrophages (*haemomacrophages, lymphomacrophages*). This term is preferable to that of mononuclear leucocytes, especially as in exudations we frequently meet with macrophages with two and even several sharply separated nuclei. Giant cells, moreover, are nothing but polynucleated macrophages. On the other hand, the leucocytes so often designated by the name of polynuclear in reality contain but a single nucleus. Even Ehrlich, who introduced this term, acknowledged its imperfection but he retained it for some time because it was already very extensively used and could, he thought, give rise to no misunderstanding. In his excellent work on anaemia, published jointly with Lazarus[1], he now agrees that the name of "cells with polymorphous nuclei" would be more exact.

[1] Ehrlich u. Lazarus, "Die Anaemie," in Nothnagel's "Specielle Pathologie u. Therapie," Wien, 1898, Bd. VIII, 1ter Theil, S. 49. Cf. the authorised English translation, "Histology of the Blood," Cambridge, 1900, p. 74.

These polymorpho-nuclear leucocytes are very numerous in the blood and in many exudations and are distinguished by the greater selective affinity of their nucleus for basic aniline dyes and by a certain tendency of the protoplasm to become stained by acid aniline colours, such as eosin. The true macrophages are without granulations, but the "polymorpho-nuclear" leucocytes contain many. These granulations are sometimes "eosinophile," "pseudo-eosinophile" (or "amphophile") or even "neutrophile" (as in man and the horse).

These two main groups of leucocytes are generally distributed in the Vertebrata; and we already meet with them in one of the lowest vertebrate forms—the *Ammocoetes* (the larva of the lamprey). The macrophages of this fish present all the principal characters of the group to which they belong (protoplasm without granules, easily stained with methylene blue, large nucleus rich in nuclear juice). In [83] the "polynuclear" forms in this lower vertebrate the protoplasm does not stain with methylene blue, but assumes a faint rosy tint with eosin; the single nucleus is divided into several lobes. In Vertebrates which are much higher in the scale these characters change. Thus in the cayman (*Alligator mississipiensis*), according to the researches of Madame Podwyssotsky, carried out in my laboratory, the two great varieties of leucocytes are readily found in the blood, lymph and exudations. The macrophages, however, especially in the exudations, are very often furnished with two or several nuclei, whilst the small leucocytes possess only a single nucleus, which is not divided into lobes. In spite of this peculiarity the two groups are readily distinguished. The staining reactions of the macrophages are identical with those of the corresponding corpuscles in all the other Vertebrata; whilst the small leucocytes, in spite of the absence of a polymorphous nucleus, are easily recognised by their eosinophile granulations and by the special affinity of the nucleus for basic aniline dyes. Under these circumstances it would be quite inappropriate to designate those leucocytes, which are really polynuclear, that is to say, possessing two or several nuclei, by the name of "mononuclear," and to reserve the name of "polynuclear" for the small corpuscles which possess only a single nucleus undivided into lobes. For this reason it is much more rational to retain for these so-called polynuclear cells my proposed name of *microphages*. Moreover, the microphages are true phagocytes. It was formerly thought that the eosinophile leucocytes, such as the "'overfed' cells (Mastzellen)" of Ehrlich, which are identical with

the *clasmatocytes* of Ranvier, never ingested foreign bodies. But, (especially after the researches of Mesnil[1]), we have been compelled to change our opinion on this point. The true eosinophile cells are able to devour foreign bodies, especially micro-organisms, and must therefore be regarded as phagocytes belonging to the group of microphages.

It is the peculiar merit of Ehrlich and of his school that they have thoroughly established the fact that, in Mammals at any rate, the two principal groups of white cells are distinguished, amongst other characters, by the diversity of their origin. The lymphocytes and the mononuclear cells are developed in the spleen and lymphatic glands, whilst the "polynuclear" cells arise from the granular mononucleated myelocytes of the bone marrow. This is now generally accepted as
[84] applicable in the great majority of cases. In *Ammocoetes*, however, the two chief varieties of leucocytes arise from one and the same organ, regarded by several observers as a kind of primitive spleen, which runs along and in part surrounds the intestine. Mesnil has been good enough to make sections of this primitive organ in which it may be demonstrated that the macrophages and the microphages in the larva of the lamprey have the same seat of origin. Frog tadpoles and Cartilaginous Fishes also possess microphages which do not arise from the bone marrow, since in them this tissue is completely absent. But even in Mammals, at least in certain pathological conditions, Dominici[2], in a research executed with much care and a perfect technique, has demonstrated the myelogenous transformation going on in the spleen. Thus in the adult rabbit affected with septicaemia by the typhoid bacillus, he found in the spleen developmental centres of amoeboid elements which, normally, appear to develop in the bone marrow only, *i.e.* the megacaryocytes, or large cells with budding nuclei, the neutrophile myelocytes (amphophiles), basophiles and eosinophiles.

The mesoblastic phagocytes of the Vertebrata are divided, then, into fixed phagocytes—the macrophages of the spleen, endothelia, connective tissue, neuroglia, and muscle fibres—and free phagocytes. These latter are sometimes haemo- or lympho-macrophages, sometimes microphages. The fixed macrophages and the free macrophages resemble one another so greatly that it is very often extremely difficult, if not impossible, to differentiate them. For this reason it is often

[1] *Ann. de l'Inst. Pasteur*, Paris, 1895, t. IX, p. 301.
[2] *Arch. de méd. expér.*, Paris, 1901, t. XIII, p. 1.

very useful, when the exact origin of a large phagocyte is not known, simply to name it "macrophage."

The two principal groups of phagocytes—(1) fixed and free macrophages, (2) microphages—are distinguished not only by their morphological characters; they also give evidence of very marked physiological differences. All phagocytes are endowed with amoeboid movement which allows them either to move about freely or merely to put out protoplasmic processes. These movements are regulated by a very great sensitiveness, often different in the two groups. Besides a tactile sense, the phagocytes possess a kind of sense of taste or chemiotaxis which enables them to distinguish the chemical composition of the substances with which they come in contact. The [85] existence of this chemiotaxis could be anticipated from the moment that an important part in the life of the organism began to be ascribed to the amoeboid cells. Leber[1], Massart and Charles Bordet[2] have, however, demonstrated it by rigorous experiment. Following the method used by Pfeffer to demonstrate the chemiotaxis of the vegetable spermatozoids and of Bacteria, these investigators introduced into the bodies of higher (rabbits and guinea-pigs) and lower (frogs) Vertebrates small glass tubes filled with different solutions (peptone, broth, salts, bacterial products, etc.). The leucocytes, guided by their positive chemiotaxis, made their way into the tubes and there formed plugs which were often very voluminous; when, on the other hand, the chemical composition of the solutions excited their negative chemiotaxis, the leucocytes avoided the tubes.

Having acquired information as to the chief characters of the leucocytes, we may ask, To which group do those amoeboid cells, which, according to the observations of Langhans and many other investigators, bring about the resorption of the red corpuscles of the blood, belong? This resorption goes on more rapidly and is observed much better if, instead of introducing blood of the same species into any part, we inject defibrinated blood, or red blood corpuscles from which the serum has been removed by washing, from another species of Vertebrate. It will be found best to inject the nucleated red corpuscles of lower Vertebrates into Mammals, or (as already described above) to introduce the non-nucleated red blood corpuscles of Mammals into lower Vertebrates. In all these cases the injection

[1] *Fortschr. d. Med.*, Berlin, 1888, Bd. VI, S. 460; "Die Entstehung der Entzündung," Leipzig, 1891.

[2] *Journ. publ. par la Soc. roy. d. Sc. méd. et nat. de Bruxelles*, 1890, 3 Feb.

of such blood or corpuscles sets up an aseptic inflammation which attracts a large number of free phagocytes to the seat of injection. In subcutaneous, peritoneal or intraocular exudations produced under these conditions, we find, in addition to a number of microphages, many macrophages. Whilst the former ingest the injected red corpuscles merely in isolated cases, the positive chemiotaxis of the macrophages manifests itself much more actively. In the resorption of the red blood corpuscles the more important part is played by the macrophage. To get a clear idea of the phenomena
[86] that accompany this resorption, let us take a concrete example. Inject defibrinated goose's blood into the peritoneal cavity of guinea-pigs[1]. During the first few hours after injection the oval nucleated red corpuscles are found intact in the fluid of the peritoneal lymph. The plasma, by itself, exercises no destructive or solvent action on the red corpuscles of the goose.

Immediately after the injection the lymph of the peritoneal cavity begins to show important changes. The white corpuscles which, in the normal condition, are fairly abundant, disappear almost completely; some small lymphocytes presenting their ordinary aspect may indeed be found, but the few macrophages and the microphages that remain show signs of very grave lesions. They lose their mobility, run together into clumps and become incapable of ingesting foreign bodies. At this moment the phagocytes undergo a critical change which we have designated by the name of *phagolysis*. This condition lasts for about an hour, sometimes it continues longer, according to case and circumstance, but after this the peritoneal fluid becomes filled with leucocytes that have newly come on to the scene. These cells make their way, by diapedesis, through the walls of the congested vessels of the peritoneum. A true aseptic inflammation is produced which induces an exudation of a large number of white corpuscles, amongst which are found microphages and still more numerous macrophages. The latter show a very pronounced positive chemiotaxis towards the injected red corpuscles of the goose. Soon after their appearance, that is to say two or three hours after the injection of the blood, the macrophages send out very small protoplasmic processes and affix them to the surface of the red corpuscles. There follows an aggregation of the macrophages of the guinea-pig with the red corpuscles of the goose and characteristic masses, in which can be recognised both kinds of cells, are produced.

[1] *Ann. de l'Inst. Pasteur*, Paris, 1899, t. XIII, p. 742.

This union with the very small pseudopodia is the first stage in the ingestion of the red corpuscles by the macrophages (Fig. 17). The red corpuscle, seized by amoeboid processes, passes into the interior of the macrophage. This macrophage seldom rests contented with ingesting a single red corpuscle. Usually it devours a large number and sometimes enormous macrophages may be seen filled with a score of red corpuscles.

If the quantity of goose's blood injected into a guinea-pig is large (5—7 c.c.), the ingestion of red corpuscles by the macrophages con- [87]
tinues for a considerable period—often for three to four days. During the whole of this time a certain number of the red corpuscles remain free in the peritoneal plasma, but, in spite of this prolonged stay, none of them undergo extracellular solution.

Fig. 17. Macrophage of guinea-pig in process of devouring and digesting red blood corpuscles of goose.

Fig. 18. Macrophage of guinea-pig in the act of ingesting and digesting red corpuscles of goose. *Intra vitam* staining with neutral red.

The red blood corpuscles, anchored by the amoeboid processes of the macrophages, at first present a normal appearance. Later their membrane begins to wrinkle, but as soon as they have passed within the phagocytes the wrinkles disappear and the corpuscles regain their normal aspect. If a little neutral red solution be added to a drop of peritoneal exudation (Fig. 18) we observe that the nucleus of the ingested red corpuscle and even its contents are stained red, whilst the red corpuscles adherent to the surface of the phagocytes retain their normal yellow colour. This reaction enables us to see that the red corpuscles are seized by the macrophages whilst still in their normal condition, but that they undergo a change immediately after they have been

ingested. Little by little the devoured corpuscles are digested within the phagocytes. The haemoglobin diffuses into the contents of the macrophage through the stroma, which has become permeable; the nucleus of the ingested red corpuscle also becomes stained by the haemoglobin. Part of this colouring matter is excreted by the
[88] phagocyte. The body of the red corpuscle is pretty soon digested, but the nucleus, impregnated with haemoglobin, persists for a much longer period. It divides into several fragments, recognisable by their yellow colour, and in certain cases these remnants of red corpuscles may be met with for weeks in the interior of the macrophages. These macrophages do not remain permanently in the peritoneal fluid. Some (3—4) days after injection the lymph of the peritoneum contains only leucocytes that have newly come up and which contain neither red corpuscles nor their remains. We must open the guinea-pig to find any macrophages that have devoured red corpuscles. They are to be met with in large numbers in the glandular portion of the omentum, in the mesenteric glands, in the liver and in the spleen. They are fairly easily recognised by the characteristic aspect of the *débris* of the red blood corpuscles. Having devoured the red corpuscles the macrophages leave the peritoneal fluid and the digestion is completed in the positions just mentioned. In the liver they are seen as large mononuclear cells often with highly developed processes. In this condition they remind one of Kupffer's stellate cells—a fact that suggested to me the idea that these elements are nothing but white corpuscles which have immigrated into the vessels of the liver.

Following up the fate of the macrophages that have resorbed the red blood corpuscles, we find them in the large hepatic vessels, in the vena cava and even in the blood of the heart. But in these latter situations they contain merely a few scarcely recognisable traces of their prey. These phagocytes, which left the blood during the inflammation that followed the injection of red corpuscles of the goose, re-enter it, having fulfilled their function, during the final period of the resorption. This resorption must undoubtedly be regarded as an intracellular digestion. When we compare the essential phenomena taking place inside the macrophages containing red blood corpuscles with those we have described in the intestinal phagocytes of the Planarians or Actinians after a meal, the analogy between the two becomes very apparent. In both cases the red blood corpuscles undergo a marked change which results in a diffusion of

the haemoglobin. The membrane and nucleus of the red blood corpuscles persist longer but they also are ultimately digested. The excretion of haemoglobin from the phagocytes, just mentioned in the case of the macrophages of the guinea-pig, is also observed in the Actinians, whose coelenteric cavity is tinted by a rose-coloured solution.

We have seen that in the Actinians intracellular digestion takes [89] place in a distinctly acid medium, whilst in the intestinal cells of the Planarians it takes place in one that is only weakly acid. The macrophages of the guinea-pig, during the resorption of red blood corpuscles of the goose, carry on the digestive process in a medium which shows a still weaker acidity. When made to ingest granules of blue litmus there is no change of colour. Nor does alizarin sulphoacid give any reaction, probably owing to the fact that it exerts a toxic action on the protoplasm of the macrophages. If, however, we add to a drop of the peritoneal exudation of a guinea-pig, containing macrophages filled with red blood corpuscles of the goose, a little of Ehrlich's 1 % solution of neutral red, the red brick tint at once makes its appearance in the content of these phagocytes. This coloration is identical with that described in the *Amoebae* which digest Bacteria or in the intestinal phagocytes of the Planarians. It may, then, be regarded as an indication of weak acidity. This coloration is maintained for some hours, after which it gives place to complete decoloration, a phenomenon that must be attributed, as in many other cases, to the neutralisation of the acid by the alkaline protoplasm that has been macerated in the fluid after the death of the macrophages.

The example we have chosen—the destruction of red blood corpuscles of the goose by the macrophages of the guinea-pig—may serve as a prototype of the resorption of formed elements in general. If, instead of red blood corpuscles of the goose, we inject into the guinea-pig's peritoneal cavity pigeon's or fowl's blood, the essential phenomena will be the same. The red blood corpuscles will always induce positive chemiotaxis, especially of the macrophages, which in turn will ingest the nucleated red corpuscles. It may be that in certain cases, when fowl's blood containing red corpuscles that are not very resistant is injected, a certain number of the corpuscles immediately undergo a partial solution in the peritoneal fluid[1].

[1] Krompecher (*Centralbl. f. Bakteriol. u. Parasitenk.*, 1te Abt., Jena, 1900, Bd. XXVIII, S. 588) has obtained a serum which was even capable of altering the nuclei of the red corpuscles of the frog. These nuclei must be much less resistant than those of the red blood corpuscles of birds, such as the goose, fowl and pigeon.

Here also the stromas and the nuclei of all the red blood corpuscles, as well as many of the corpuscles unacted upon by the plasma of the phagolysed exudation, undergo digestion inside the macrophages.

[90] When, instead of blood, we inject white corpuscles from the bone marrow, spleen or lymphatic glands of animals into the peritoneal cavity, we may still observe their final disappearance in the macrophages. The spermatozoa of man or of various mammals (bull, rabbit, guinea-pig, etc.), when injected into the peritoneal cavity of the guinea-pig or rabbit, are well adapted for this line of investigation. Here again the immediate result of injection is the very marked phagolysis of the leucocytes. This phenomenon gives place to an exudative inflammation which brings into the peritoneal cavity a number of phagocytes. These, especially the macrophages and in a much smaller degree the microphages, devour the spermatozoa which in no case are dissolved, even partially, in the plasma of the exudation. The macrophage seizes the spermatozoa which sometimes, by the active movements of their flagella, exhibit great vitality. At the end of several hours all the spermatozoa are found inside phagocytes where they are completely destroyed. The flagellum is digested first, but the head and medial portion soon suffer the same fate. Neutral red reveals the feebly acid reaction, perhaps with even more distinctness than in the case of the red blood corpuscles.

The *résumé* of Langhans' investigation given in this chapter would lead us to expect that resorption in the subcutaneous tissue will follow the same rules as that going on in the peritoneal cavity. As a matter of fact, blood injected at this position sets up a diapedesis of phagocytes which ingest the red blood corpuscles. In some cases only is there a partial solution of these corpuscles in the fluid of the subcutaneous exudation. It is for this reason that goose's blood, injected under the skin of a guinea-pig, gives rise to a fluid exudation coloured a bright rose red by the dissolved haemoglobin. This haemoglobin is derived from red blood corpuscles which are damaged by the goose's blood serum that was added to the plasma of the exudation. The stroma and nuclei of the red blood corpuscles cannot, however, be dissolved in this fluid. They undergo the same fate as the red corpuscles that have remained intact, that is to say they are ingested by the macrophages which immigrate into the subcutaneous tissue and which finally digest all these elements. The cells, less fragile than certain red corpuscles, are, in the subcutaneous tissue, as in the peritoneal cavity, destroyed solely in the interior of the phagocytes.

The analogy between the modifications undergone by the red blood corpuscles and other cells inside the macrophages and the changes that take place in the intestinal cells of Planarians and Actinians, suggests that the resorption of formed elements must [91] undoubtedly be regarded as a true intracellular digestion. It would, however, be a very important matter to be able to support this conclusion by even more convincing proofs. The study of the artificial digestion that is observed *in vitro* in the case of the macerated mesenterial filaments of Actinians has furnished a very valuable argument in favour of the enzymatic nature of intracellular digestion. Animal exudations are not well adapted for this special line of study. We can only obtain them as the result of the injection of different substances, solid or fluid, which are greedily absorbed by phagocytes. If we collect the exudations at a moment, when the number of these cells is still considerable we must withdraw along with them many digestive substances which interfere with our observation. We may therefore with advantage turn our attention to masses of phagocytes collected in organs. As it is mainly the macrophages which effect the resorption of cells, it is evident that we must choose the centres where they are formed in order to investigate the digestive ferments. Let us take, then, the lymphatic glands of the mesentery, the glandular portion of the omentum and the spleen, the three pre-eminently macrophagic organs, and let us see if, with an extract of them, prepared with physiological salt solution (0·75 % of sodium chloride), any digestive effect is to be obtained.

Macerate the three organs mentioned of a guinea-pig and mix the extracts thus obtained with red blood corpuscles of the goose, corpuscles that have already given us information in connection with the phenomena of resorption in the living organism. In almost all the guinea-pigs a solution of the red blood corpuscles of the goose by the extract of the glandular portion of the omentum may be observed. The mesenteric glands likewise give an extract which in most cases has a solvent action. The extract from the spleen is only active in a limited number of cases. In all these examples the extracts from macrophagic organs bring about the solution of the haemoglobin, but leave intact the membrane and nucleus of the corpuscles. In this respect there exists, then, a certain difference between this and the digestion of red corpuscles in the macrophages of exudations, where the membrane and even the nucleus are in the end completely dissolved. This difference may be explained by the

fact that in the preparation of the extract in physiological salt solution, one part only of the soluble digestive ferment may be set at liberty.

The solvent action of extracts of macrophagic organs must in fact
[92] be attributed to the presence of a soluble ferment in the cells of which these organs are made up. As the diastases are distinguished, in general, by their great sensitiveness to heat, we tried the action of our extracts after a preliminary heating, when it was found that a temperature of 56° C., applied for three quarters of an hour, completely abolished the solvent action of the extracts upon the red blood corpuscles of the goose. The soluble ferment of macrophagic organs, to which we propose to give the name of *macrocytase*[1] or macrophage ferment, is in many respects analogous to the actino-diastase of Mesnil, described in the preceding chapter.

With a view to obtain more complete information on the cytases I suggested to Tarassewitch that he should make a detailed study of them; this he has carried out in my laboratory. He has demonstrated that the macrophagic organs of other mammals than the guinea-pig, especially those of the rabbit and dog, exert the same solvent action on the red blood corpuscles. He has also established the fact that this action applies not only to the red corpuscles of the goose but extends also to those of several other birds and mammals. Tarassewitch succeeded in confirming the injurious action of heat on macrocytase. Extracts of macrophagic organs which contain much *débris* in suspension, when heated for an hour at 55°·5 C. in certain cases lose their solvent property for red blood corpuscles; sometimes this temperature brings about merely a weakening of the macrocytase. In order to destroy it surely and completely, the suspensions must be heated at 58°·5—62° C. for an hour. If, however, instead of heating the entire suspension, we first pass it through filter paper, the clear fluid filtrate is deprived of its diastatic action even after it has been heated at 55°·5 C. for three quarters of an hour.

Of all the other organs of which extracts have been kept in prolonged contact with the red blood corpuscles of birds, the pancreas alone has shown a very well-marked digestive action. Extracts of the

[1] Some years ago it was proposed to give the name of cytase to the ferments which digest cellulose. Thus Laurent, in the work analysed in the second chapter, applies it to the ferment secreted by the bacilli which attack the vegetable membrane. We think that the cellulose ferment should be designated by the name of *cellulosase* and that the name of cytase would be more suitable for a soluble ferment which digests the cells.

salivary glands exerted a feeble solvent action on a certain quantity of the red corpuscles. The other organs, such as the liver, kidneys, [93] brain, spinal cord, ovary, testicles, suprarenal capsules and placenta, exercised no such action. Even bone marrow, in agreement with my results published some years ago, showed itself quite inactive.

The blood serum of guinea-pigs which I employed in my researches, as well as that of the animals examined by Tarassewitch, has not shown itself capable of dissolving the red blood corpuscles of the goose, although the macrophagic organs dissolve them easily. It has long been known, however, that the serum of the blood of many animals will destroy the red corpuscles of a different species. This demonstration was afforded during the period when attempts were being made to transfuse the defibrinated blood of mammals, especially of the sheep, into man. This practice had to be abandoned, in consequence of the difficulties resulting from the solution of the human red corpuscles. Later, Daremberg[1] and Buchner[2] set themselves to study this haemolytic action of serums systematically. They found that it was due to a particular substance to which Buchner gave the name of *alexine* or protective substance. Of indeterminate chemical composition, this substance is allied to albuminoid substances. It is destroyed when heated to 55°—56° C. and only acts in the presence of certain salts. When these salts are removed from the serum by dialysis, it loses its haemolytic power; but as soon as the salts are replaced in proper proportion this power reappears. Later, Buchner[3] compared the action of alexine to that of soluble ferments and referred it to the category of the digestive diastases. According to him the same alexine is capable of dissolving the red blood corpuscles of several species of Vertebrates. Bordet[4], in a series of researches made in the Pasteur Institute, confirmed this view. He came to the conclusion that the alexines of the various species of animals differ from one another. Thus, the alexine of the blood serum of the rabbit is not the same as that found in the serum of the guinea-pig or dog. Nevertheless each of these alexines is capable of exerting a solvent action on the red blood corpuscles of several species.

[1] *Arch. de méd. expér.*, Paris, 1891, t. III, p. 720.
[2] *Verhandl. d. X. Congr. f. inn. Med.*, Wiesbaden, 1892.
[3] *München. med. Wchnschr.*, 1900, S. 1193.
[4] *Ann. de l'Inst. Pasteur*, Paris, 1899, t. XIII, p. 273; 1901, t. XV, p. 312.

[94] Ehrlich and Morgenroth[1], in a series of memoirs on the solution of red blood corpuscles, have combated the idea that there is only a single alexine in one and the same serum. Moreover, they state that alexine always requires for its action the aid of another substance, and that matters are much more complicated than at first sight appears. They maintain that in each normal serum a number of different substances are found, each one of which only attacks a single species of red blood corpuscle. They point out that the solution of the red corpuscles by the normal serum takes place through the combined action of two different substances and cite several cases where a normal serum, after being heated to 55° C. and so deprived of its haemolytic power, again becomes capable of dissolving the red corpuscles when some normal serum from another species, which of itself is destitute of the solvent property, is added to it. Let us quote an example from Ehrlich and Morgenroth. The normal serum of the goat readily dissolves the red blood corpuscles of the rabbit and guinea-pig, but if heated for half an hour at 55° C., it loses this power. On the other hand, the normal serum of many horses shows itself powerless to dissolve the red corpuscles of these rodents. Here, then, are two serums, equally incapable of effecting the solution of the red corpuscles of the rabbit and guinea-pig. Yet, when they are mixed together and to them a few drops of blood from one of the rodents cited is added, haemolysis takes place readily. The heated goat's serum then, has, retained in it something that resists a temperature of 55° C., a substance which, by itself, leaves the red blood corpuscles intact; but which, when combined with a second substance present in the horse's serum, causes their solution. Ehrlich gives to the first substance, that is to say that found in the heated goat's serum, the name of *intermediary body* ("Zwischenkörper"). The second substance, present in the unheated horse's serum, is designated by him the *complement*. In order that a normal serum may dissolve the red corpuscles, it is not sufficient that it should possess a single substance, the alexine of Buchner. It must, to exert this action, contain two distinct substances which are very often found together in the same normal serum. Unheated goat's serum was only capable of dissolving the red blood corpuscles of the rabbit because a particular complement and intermediary substance were both present. Deprived of its complement at 55° C., the serum is solvent only when we add to it another
[95] substance that is contained in the normal serum of a different species

[1] *Berl. klin. Wchnschr.*, 1899, SS. 6 and 481.

(horse). Continuing their researches in this direction, Ehrlich and Morgenroth have come to the conclusion that the normal serum of a single species may contain several intermediary substances, each one acting on a single species of red blood corpuscles. Further, that normal serum must contain several or even many different complements.

Ehrlich and Morgenroth carried on researches on the intermediary substances in normal serums and found several in addition to that already mentioned. The serum of the normal dog readily dissolves the red blood corpuscles of the guinea-pig. When heated to 57° C. it loses this property; but with the addition of normal guinea-pig's serum the property is regained. In the serum of the normal dog there exists, then, besides the complement, at least one intermediary substance. The same result can be obtained with several combinations of serums of normal mammals, heated or unaltered[1]. Yet it often happens, as Ehrlich and Morgenroth themselves point out, that the demonstration of the presence of the intermediary substance in normal serums is accompanied with marked difficulties. Bordet, also, who has studied this question very thoroughly, often failed completely in his attempts to make normal serums, that were incapable of producing haemolysis, active by the addition of heated serums of other species of animals. Thus he observed that normal fowl's serum readily dissolves the red corpuscles of the rabbit. When heated to 55°—56° C. this serum loses its haemolytic power which cannot be restored by the addition of any normal serum. He thinks therefore that, in this example, haemolysis is produced solely by the alexine, without the assistance of any intermediary substance in the serum of the normal fowl. P. Müller[2], whilst confirming Bordet's experimental results, considers that, in this case also, there is the intervention of an intermediary substance. When he mixed heated fowl's serum with a small quantity of unaltered fowl's serum the solution of the red corpuscles of the [96] rabbit is not brought about. When, however, instead of adding a little unheated normal fowl's serum, he added the same quantity of

[1] Ehrlich and Morgenroth, "Ueber Haemolysine," II, *Berl. klin. Wchnschr.*, 1899, S. 481. The following are the combinations found by these observers: heated calf's serum with normal serum dissolves the red blood corpuscles of the guinea-pig; heated rabbit's serum plus sheep's serum dissolves the red blood corpuscles of the sheep; heated serum of rabbit with the addition of goat's serum dissolves the red corpuscles of the goat; heated sheep's serum with guinea-pig's serum produces haemolysis of the red corpuscles of the guinea-pig.

[2] *Centralbl. f. Bakteriol. u. Parasitenk.*, I^te^ Abt., Jena, 1901, Bd. XXIX, S. 175.

serum from a fowl previously treated with physiological salt solution, the red corpuscles of the rabbit were dissolved without any difficulty. Müller explains this difference as due to the fact that the serum of the treated fowl contains more complementary substance than does that of the normal fowl.

We see, then, from this example that the analysis of the phenomena taking place in the solution of the red corpuscles by normal serums is beset with very great difficulties. For this reason it is much more profitable to make researches in this direction, using more active serums, where the demonstration of the two substances can be made simply and exactly. This desideratum has been supplied by J. Bordet, when *preparateur* in our laboratory; he described an easy method of increasing the haemolytic power of serums.

As stated above, guinea-pigs that have received an intraperitoneal injection of goose's blood digest the corpuscles, although the peritoneal fluid exerts no haemolytic action. *In vitro*, the extract of their macrophagic organs certainly dissolves the red corpuscles, whilst the blood serum usually fails to do so. Now, if a second or a third injection of goose's blood be made into the peritoneal cavities of the same guinea-pigs, partial solution of the corpuscles takes place in the peritoneal plasma and the serum of the blood acquires new properties: it becomes capable of clumping the red corpuscles, that is to say of agglutinating them; afterwards it dissolves them *in vitro*.

J. Bordet[1] has shown that the injection of the blood of one species of Vertebrate (mammal or bird) into the peritoneal cavity or under the skin of an animal of a different species, always produces in the blood serum of the latter the haemolysing substance. This haemolysing substance is specific or nearly so, that is to say it dissolves the red corpuscles of the species which has furnished the injected blood and also, but more feebly, the red corpuscles of allied species. Consequently, with guinea-pig's serum, treated with goose's blood, we obtain the greatest solvent action on the red corpuscles of the goose, though there is a certain haemolysis of the red corpuscles of some other birds. This rule, thoroughly established by Bordet, has been the starting-point for a large number of researches on haemolysis
[97] and amongst others of those which bear on the intermediary substance of normal bloods.

Bordet demonstrated very definitely a fact of fundamental importance—that in the blood serums of animals treated with blood from a

[1] *Ann. de l'Inst. Pasteur*, Paris, 1898, t. XII, p. 688.

different species, there exist two distinct substances which only dissolve the red blood corpuscles when they are combined. Here the duality of the haemolytic agent cannot be doubted, as it may in certain examples of normal serums. Each time that we deprive the serum of a treated animal of its solvent action by heating at 55°—56° C., this property can be restored to it with certainty by the addition of a little normal serum which, by itself, is incapable of bringing about haemolysis. The heated serum of these injected animals loses the power of dissolving the corresponding red corpuscles, but it retains its other acquired property—the agglutination of the corpuscles. The red corpuscles, aggregated into voluminous masses quite visible to the naked eye, remain intact indefinitely, if left in the prepared and heated serum. But as soon as we add to them a trace of normal blood (taken from one of a number of species of Vertebrates), the solution of the red corpuscles is not long in taking place. Under these conditions an action of two substances is set up; one of these substances is found in the heated serum of the injected animal, and the other in unheated normal serum. The first of these substances which not only resists a temperature of 55°—56° C., but stands, without alteration, heating to 60°—65° C., corresponds to the intermediary substance of Ehrlich. By Bordet it has been termed "substance sensibilisatrice[1]." The second substance, a common one, found in normal serums and destroyed at 55°—56° C., is the alexine of Buchner and of Bordet, or the complement of Ehrlich.

The ease with which one can demonstrate the co-operation of two substances in the haemolysis by the serums of animals treated with the blood of a different species, is due to the fact, that during the course of this treatment the animal organism produces a quantity of an intermediary or sensibilising substance. In fresh animals which have not been treated, it is often very difficult to demonstrate the presence of this substance. Bordet has established the fact that [98] the serum of animals which have been injected several times with the blood of a different species, contains almost the same amount of alexine as does untreated serum. On the other hand, the sensibilising substance makes its appearance in large quantity as the result of these injections. Von Dungern[2] has confirmed this result and con-

[1] Among the synonyms of this substance, resistant to the action of heat, we may mention the following: haemolytic antibody, preventive substance, immunising body (Immunkörper of Ehrlich), amboceptor (Ehrlich), philocytase (Metchnikoff), desmon (London), copula (P. Müller).

[2] *München. med. Wchnschr.*, 1900, S. 677.

tributes the interesting fact that the sensibilising substance is found even in great excess in the serum of treated animals. When he adds to this serum blood that has not been heated, he produces a haemolysis that is more than thirty times as active as when the serum of the prepared animal alone is used. From the quantitative point of view, then, there is no relation between the amount of the two substances in the serum of prepared animals.

It may be suggested that the sensibilising or intermediary substance is the same as that which produces the agglutination of the red corpuscles. But careful researches have thoroughly demonstrated the difference between the two substances that have this character in common, both resist heating to 55°—60° C. and even beyond this point.

Having established this co-operation of two substances in haemolysis the intimate mechanism of their action was next studied. Here I must give pride of place to the discovery by Ehrlich and Morgenroth that the intermediary (or sensibilising) substance links itself to its corresponding red corpuscles. A serum, capable of dissolving the red corpuscles of a different species, is heated to 56° C. which causes it to lose this solvent property. When a certain number of these corpuscles are added to it, such corpuscles remain intact although they are agglutinated. It is sufficient, after some hours of contact, to centrifugalise the mixture in order to separate a limpid serum from the mass of red corpuscles, the former being now entirely deprived of its intermediary substance, that is to say it has become incapable of dissolving the red corpuscles even with the addition of a large quantity of the "complement" (normal serum, unheated). On the other hand, the red corpuscles, having fixed (linked) all the intermediary substance, dissolve very rapidly when placed in contact with normal serum which contains the necessary quantity of the complement (or alexine). This fundamental experiment has been confirmed and repeated by many observers and has now become classic. The idea that the intermediary (or sensibilising) substance links itself to the red corpuscle, without dissolving it, is generally accepted and may be regarded as permanently settled. We should do well, then, instead
[99] of designating by all sorts of synonyms the substance in serums which resists the action of a temperature of 55°—65° C., to apply to it, once for all, the name of *fixative substance* or simply that of *fixative.* This name is short, expresses the essential character of the substance and gives rise to no misunderstanding, as do the other names proposed up to the present (amongst them that of *philocytase* employed by myself in some of my earlier publications).

Another of Ehrlich and Morgenroth's experiments has furnished the proof that the complement does not fix itself to the red corpuscles only. A normal serum, unheated, which, by itself, is quite as incapable of dissolving the red corpuscles as the fixative alone, is mixed with some defibrinated blood. After the centrifugalisation of this mixture, it is easy to demonstrate that the supernatant fluid has lost none of its complement (alexine), whilst the red corpuscles have fixed none.

If, instead of an inactive serum, we take a serum which is capable of dissolving the red corpuscles and which consequently contains the two haemolysing substances, and if we place it in contact with the corresponding red corpuscles, at a temperature between 0° and 3° C., the solution will not take place (Ehrlich and Morgenroth). Under these conditions the fixative certainly attaches itself to the red corpuscles, but the alexine remains in solution, unused. It is only necessary, however, to heat the mixture up to 30° C. to bring about rapid haemolysis.

From their very ingenious experiments, as a whole, Ehrlich and Morgenroth conclude that the fixative possesses two different affinities: one for the red corpuscle and another for the complement. Of these two affinities the stronger is that which links it to the red corpuscle, for this is manifested at a very low temperature. In order that the fixative may combine with the complement a much higher temperature is requisite. Ehrlich comes to the conclusion that the molecule of the fixative possesses two haptophore groups, or groups capable of chemical combination. The first of these links it to a corresponding molecule of the red corpuscle to which he gives the name of receptor; the second combines the fixative with the molecule of the complement and in this way introduces the latter into the red corpuscle. These investigators give a diagram which greatly facilitates the understanding of their hypothesis (Fig. 19). They seek to prove that the combinations of the fixative with the red blood corpuscle and with the complement follow the law of definite multiples and that these phenomena must, in consequence, [100] be looked upon as being of a purely chemical character.

Fig. 19. Schema of Ehrlich's theory.

c, complement (alexine, cytase) — *am*, amboceptor (fixative) — *r*, receptor of the red corpuscle.

(After Levaditi in the *Presse médicale*.)

The hypothesis advanced by J. Bordet does not accord very well with the theory we have just set forth. He could never convince himself that the fixative combines with the complement. He was of opinion rather that the fixative, retained by the corpuscle, exercises upon it a mordant action which enables it to absorb the alexine. The alexine is supposed to attach itself to the sensibilised red blood corpuscle as a dye attaches itself to a mordanted element. Bordet rests his interpretation mainly on the fact that the absorption of alexine by the sensibilised corpuscles does not follow the elementary laws of chemical combination, especially those of definite multiples.

Nolf[1] has sought to define more accurately the part played by these two substances in the solution of the red blood corpuscles. He agrees with Bordet, that in this phenomenon the fixative plays the same part that the mordants do in dyeing. Linked to the red corpuscle the fixative renders it more greedy for alexine, exactly as the mordant facilitates the fixation of the dye on the fibre of the textile fabric. Under these conditions the alexine (complement), finding itself in large quantity inside the red corpuscle, exercises upon it its hydrating action, thus bringing about the diffusion of the haemoglobin and often even the solution of the corpuscular stroma.

Nolf compares the solvent action of alexine upon the red corpuscle to that of certain mineral salts, such as ammonium chloride. He passes in review the various properties of alexines and finds them very similar to the solvent action of certain salts. Even the peculiarity of alexine, of remaining inactive at a temperature of 0°—3° C., is shared by ammonium chloride which, alone of all the salts studied by Nolf, exercises no solvent action under these conditions. But Nolf found it impossible to push these analogies further, and especially to sensibilise, by the fixative, the red corpuscles to the action of quantities, which were of themselves inactive, either of ammonium chloride or of any other salt.

[101] London[2] hoped by fresh experiments to solve the problem of the mode of action of the two substances which act in haemolysis. He pronounced in favour of the theory that they entered into chemical combination with the red corpuscles. But the facts accumulated up to the present do not enable us to make a positive statement as to the exact nature of the reaction which is set up during the

[1] *Ann. de l'Inst. Pasteur*, Paris, 1900, t. XIV, p. 656.
[2] *Arch. d. sc. biol.* (russes), 1901, t. VIII, pp. 281 and 323.

solution of the red blood corpuscles; this is not astonishing in view of the fact that it is impossible to isolate the haemolysing substances in a pure state.

It may, however, be admitted that the action of alexine (complement) comes under the category of phenomena that are produced by soluble ferments. Buchner[1] maintains that there is an analogy between this substance and the diastases (or enzymes); Bordet[2], from the appearance of his first publications on haemolysis, has expressed himself in favour of this view. Ehrlich and Morgenroth[3], in their two first memoirs, very distinctly put forward the same idea. "We shall not deceive ourselves"—they say—"if we attribute to the addiment (syn. complement, or alexine) the character of a digestive ferment." In one of their last memoirs[4] they no longer express themselves in so decided a fashion. Nevertheless we are still quite justified in maintaining this proposition. The substance which dissolves the red blood corpuscles of Mammals or a portion only of those of Birds, undoubtedly presents very great analogies to the digestive ferments. As has been mentioned repeatedly, it is very sensitive to the action of heat and is completely destroyed by heating for one hour at 55°C. In this respect it closely resembles the macrocytase of macrophagic organs which also dissolves red corpuscles. As it is the macrophages which ingest and digest the red blood corpuscles in the organism, it is evident that alexine is nothing but the macrocytase which has escaped from the phagocytes during the preparation of the serums.

We know that the leucocytes contain quite a series of soluble ferments of which some are set at liberty after the blood has been drawn from the vessels. It is thus that plasmase, or fibrin-ferment, is set free from the leucocytes to combine with fibrinogen to produce [102] the clot. This is not the only soluble ferment of leucocytic origin. It has been known for some time that in addition to this coagulating ferment the leucocytes contain ferments which are especially digestive or decoagulating. Thus Rossbach[5] has demonstrated the presence of amylase in the leucocytes of different organs, especially the tonsils. Arthus has confirmed this discovery and Zabolotny[6] has completed it by his observations on the phenomena which appear in the peritoneal

[1] *München. med. Wchnschr.*, 1900, S. 1193.
[2] *Ann. de l'Inst. Pasteur*, Paris, 1898, t. XII, p. 688; 1899, t. XIII, p. 273.
[3] *Berl. klin. Wchnschr.*, 1899, SS. 6 and 481.
[4] *Berl. klin. Wchnschr.*, 1900, S. 682.
[5] *Deutsche med. Wchnschr.*, Leipzig, 1890, S. 389.
[6] *Arch. russes d. path.*, etc., St.-Pétersb., 1900, t. IV, p. 402.

cavity of animals into which wheat flour or starch were injected. He observed that the small granules are quickly ingested by isolated leucocytes, whilst the large granules are surrounded by quite a layer of phagocytes. He agrees with several other writers, that the amylase found in defibrinated blood has its origin in leucocytes.

Leber[1], in the course of his researches on inflammation, made the observation that the pus of a hypopyon that was absolutely aseptic digests coagulated fibrin at a temperature of 25°C. and liquefies gelatine very readily. Achalme[2] has confirmed this and has added several other interesting data. He investigated the soluble ferments of pus and directed his attention amongst others to experimental pus, set up by the injection of spirit of turpentine. In addition to amylase and a ferment which liquefies gelatine, Achalme has discovered in pus, saponase (lipase), casease, and a ferment closely allied to trypsin. This last readily digests fibrin and also attacks coagulated white of egg; in the products of this digestion Achalme found peptone but could not always obtain leucin and tyrosin. He never succeeded in demonstrating the presence of sucrase, inulase, emulsin or lactase in pus. On the other hand he found large quantities of oxydase, thus confirming the discovery of Portier[3] who was the first to demonstrate that these ferments met with in the blood are, in the living animal, found inside leucocytes. By a large number of experiments,
[103] carried out on most diverse representatives of the animal kingdom, Portier was able to establish the important fact that the oxydases which are found in many organs or in the fluid of blood withdrawn from the organism really originate in leucocytes as they deteriorate and break up. In this respect, then, they resemble fibrin-ferment very closely.

To complete the list, already considerable, of leucocytic ferments, I must further cite the anticoagulating soluble ferment whose existence in Mammals has been so well demonstrated by Delezenne.

All this evidence encourages us, then, to support the thesis that alexine is one of the numerous intraleucocytic soluble ferments and that it only passes into the fluids as the result of rupture or of damage to the phagocytes. Nolf (*l. c.*) has recently pronounced against this view; we must therefore examine his arguments closely. In the first place he takes his stand on the analogies between the

[1] "Die Entstehung der Entzündung," Leipzig, 1891, S. 508.
[2] *Compt. rend. Soc. de Biol.*, Paris, 1899, p. 568.
[3] "Les Oxydases dans la série animale," Paris, 1897.

solution of the red blood corpuscles by the serums and by certain salts. It must not be forgotten, in connection with his theory, that haemolysis is but one example, out of many, of the action of alexines. Of all the formed elements the red corpuscles are the most delicate; they are readily broken up by all sorts of agents (moderate heat, water, salts, etc.). Further, there are numerous other cells (white corpuscles, spermatozoa, and inferior organisms) which resist the action of salts much better, which, nevertheless, are very injuriously affected by the action of the alexines.

Nolf lays special stress on the experiments in which, after keeping red blood corpuscles in prolonged contact with active serums, he has looked in vain for the peptone reaction. He prepared his mixtures in sealed tubes or flasks, and kept them in an incubator at 37° C. for 24—48 hours, or even for weeks. Under these conditions the haemoglobin is transformed into metahaemoglobin, but peptones never appear. Nolf concludes therefrom "with confidence, that the alexines do not exert the slightest peptonising effect on the albuminoids of the corpuscle" (*l. c.* p. 672).

To this conclusion it must be objected that peptone is not the only product of the digestion of albuminoids by soluble ferments. Under certain conditions the disintegration is carried much further, in others it is arrested at an earlier stage. Thus human urine which contains pepsin, never gives the peptone reaction with fibrin; the digestion of the latter only goes on up to the stage of protalbumose. When, however, the urinary pepsin is fixed on flakes of heated fibrin [104] which are submitted to digestion in acidulated water the digestion proceeds further and gives as final products deuteroalbumose and peptone[1]. Now, under the conditions in Nolf's experiments the digestion would be very quickly stopped, because, at the temperature of 37° C., alexine very soon loses its strength. Investigators who have experimented with haemolytic serums know well that, even when kept at a low temperature, alexine may lose its activity within 24 hours.

It has been mentioned above that Nolf sought in vain for a parallel between haemolysis by salts and that by serums, in what relates to the action of the fixative. He was unable to find anything comparable to this action amongst salts, although digestion by soluble

[1] Stadelmann, *Ztschr. f. Biol.*, München, 1887, Bd. XXIV, S. 226; 1888, Bd. XXV, S. 208; Patella, *Ann. univ. di med. e chir.*, Milano, 1887. (Cited by Huppert in Neubauer u. Vogel's *Analyse des Harns*, X^te^ Aufl., Wiesbaden, 1898, S. 599.)

ferments offers undoubted analogies. I need only recall further the discovery of enterokynase, the soluble ferment of the digestive juice of the dog, which actively stimulates the action of pancreatic ferments, and especially that of trypsin. The recent researches of Delezenne (communicated to the International Congress of Physiology held at Turin in September 1901) support this conclusion in a very important fashion. As already pointed out in Chapter III the enterokynase of the intestinal juice exerts an action comparable with that of the fixatives of haemolytic serums. Alone, it does not act as a solvent ferment, but when it attaches itself to the fibrin it aids the action of the trypsin in a marked degree. In pancreatic digestion enterokynase plays the part of the fixatives in the solution of red corpuscles.

The analogy between the resorption of formed elements and intestinal digestion extends even beyond this. When we inject, into the peritoneal cavity or under the skin of various animals, blood from a different species, the blood serum of the former becomes haemolytic for the red corpuscles of the latter. The solution of these red corpuscles is effected by the alexine of the serum, whose activity is rendered very great owing to the presence of a quantity of specific fixative. This same fixative appears also in the fluids of animals to whom, instead of injecting blood, we simply give it by the mouth.
[105] This fact has been established by Metalnikoff[1].

Another fact in favour of the close relationship between the fixatives and enterokynase consists in the presence of both in the lymphatic (lymphopoietic) organs. The fixatives which aid the solution of red corpuscles are found specially in the mesenteric glands. Enterokynase, as demonstrated by Delezenne, is found not only in the intestinal juice, but also in Peyer's patches, the solitary glands, the mesenteric glands, and the leucocytes of exudations and of the blood.

Supported by these various facts we are quite justified in regarding the haemolysing substance of serum as containing two soluble ferments, of which one, alexine, corresponds to trypsin, the other, the fixative, resembling enterokynase. The alexine, whose nature is gradually disclosing itself with more precision, should bear the name of *cytase* or cell-ferment. The cytase of the macrophagic organs, or *macrocytase*, comes under this category. According to the researches of Tarassewitch it also acts more actively when there is added to it some of the fixative found in the serum (heated to 56° C.) of prepared animals.

[1] *Centralbl. f. Bakteriol. u. Parasitenk.*, I^te^ Abt., Jena, 1901, Bd. XXIX, S. 531.

We have said that in the living animal the macrocytase is localised in the phagocytes of the organs and of the blood. Thus, when goose's blood is injected into the peritoneal cavity of the guinea-pig the red blood corpuscles are digested within the macrophage and not in the fluid of the peritoneal exudation. When, however, the same kind of blood is injected a second or a third time, it is found that a certain number of the red corpuscles become permeable and lose their haemoglobin, which they give up to the fluid of the exudation, and only the membrane and the nucleus remain. These are at once ingested by the macrophages which under these conditions manifest a real excess of activity. Instead of sending out small processes, as they do after the first injection of blood, these phagocytes move about like true Amoebae, sending out broad pseudopodia, and ingest not only the remains of the red corpuscles but also those still intact[1] (Fig. 20). Under these con- [106]
ditions macrocytase must undoubtedly be found in the peritoneal plasma. It is, however, easily demonstrable that this ferment was not preformed in the fluid but has escaped from the leucocytes that have undergone phagolysis. After the rapid injection of alien blood the phagocytes of the peritoneal lymph gather into clumps, become immobile, and for a time lose their phagocytic power. It is only after the lapse of a longer or shorter period that the leucocytes recover from the phagolysis, arrive in great numbers in the peritoneal cavity and display their phagocytic energy.

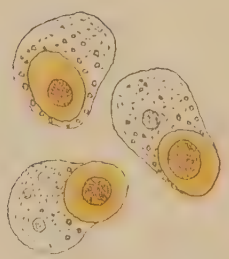

Fig. 20.—Rapid ingestion of red corpuscles of the goose by macrophages.

If the damage to the phagocytes—the phagolysis—is the actual cause of the setting free of the intraleucocytic ferment, we have only to prevent this phagolysis in order to inhibit the solution of red blood corpuscles in the fluid of the exudation. For this purpose it is sufficient to prepare guinea-pigs (which have already received several injections of goose's blood) by means of an injection of fresh broth, of physiological salt solution, or of carbonic acid into the

[1] Sawtchenko (*Arch. russes de Path.*, etc., St. Pétersb., 1901, t. XI, p. 455) has observed that leucocytes, after they have absorbed the specific fixative, acquire the property of ingesting red blood corpuscles with extraordinary rapidity. Tarassewitch was able to confirm this fact.

peritoneal cavity on the eve of the decisive experiment. Such injection at once provokes phagolysis, which is then followed by an abundant exudation of leucocytes. When, next day, a dose of red blood corpuscles of the goose (deprived of serum by centrifugalising) is introduced into the peritoneal cavity thus prepared phagolysis is no longer produced, or very feebly, and is of very short duration. Under these conditions the solution of the red corpuscles by the peritoneal fluid is reduced to a minimum, and in its place an extremely rapid and considerable ingestion of red corpuscles by the macrophages may be observed. In order that the experiment may be completely successful it is advisable to use goose's blood heated to 37°C. or thereabouts for the injection.

Even when the red corpuscles of the goose are introduced, not into the peritoneal cavity but into the subcutaneous tissue of guinea-pigs that have received several injections of goose's blood, we can easily prevent the extracellular solution of the red corpuscles which takes place, as already indicated, in the normal guinea-pig. As in this
[107] case the goose's serum which is mixed with the corpuscles contributes to the haemolysis, it must be suppressed by centrifugalising the defibrinated goose's blood and by washing the corpuscles with normal saline solution.

Collectively, the facts I have just described clearly indicate that the phagocytes must be regarded as the source of the haemolytic ferment. The macrocytase remains in the body of these cells so long as they are in a normal condition; but immediately they are injured, in consequence of the sudden introduction of foreign substances into the peritoneal cavity, a portion of the macrocytase escapes and acts on the red corpuscles as if it had been employed *in vitro*.

As the conclusion I have just formulated is of fundamental importance in the study of resorption and immunity it is necessary to support it by as many arguments as possible. For this reason, therefore, I feel obliged to draw the attention of the reader to another example of the resorption of formed elements.

We have already spoken of the resorption of spermatozoa in the peritoneal cavity, and of the part played by the macrophages in this phenomenon. As a result of this resorption, just as after that of red blood corpuscles, the organism acquires new properties of the same character. Landsteiner[1] and the writer[2] have shown

[1] *Centralbl. f. Bakteriol. u. Parasitenk.*, I^te^ Abt., Jena, 1899, Bd. XXV, S. 546.
[2] *Ann. de l'Inst. Pasteur*, Paris, 1899, t. XIII, p. 738.

that the blood serum and the peritoneal fluid of animals that have been injected with the spermatic fluid of bull, rabbit, or man, become spermotoxic, that is to say, they render the corresponding spermatozoa motionless and kill them. These fluids, however, never acquire the power of dissolving, even partially, these elements. The disappearance and final solution of the spermatozoa is only effected within phagocytes, and almost exclusively in the macrophages.

Moxter[1] has demonstrated that the spermotoxin which appears in the serum of prepared animals consists of two substances, corresponding to those present in the haemolytic serums. These are the macrocytase (alexine, complement) and the fixative (intermediary or sensibilising substance). For him they are identical with those which dissolve the red corpuscles. Without dwelling on the subject we may say that the macrocytase which dissolves the red corpuscles and that which arrests the motion of the spermatozoa are really identical in the same species of animal, as is accepted and developed [108] by Bordet. On the other hand, it is impossible to accept Moxter's theory of the identity of the two fixatives. They must be regarded as different; this we have attempted to prove in one of our memoirs[2] and is in accordance with the law of the specificity of fixatives in general.

The question which interests us more especially at this moment is where are these two constituent substances of the spermotoxin to be found and how do they behave in the living organism? This question has been very thoroughly studied by Metalnikoff[3] in my laboratory. His experiments have been closely followed by me, and in presenting their principal results I can bear witness to their correctness.

The spermotoxin obtained by Metalnikoff is distinguished from the haemotoxins we have discussed up to the present in that they develop, not as a result of the injection of cell elements from a different species, but as a result of the introduction into the organism of spermatozoa from the same species, the guinea-pig. We have here, then, to deal with what has been termed autospermotoxin.

The serum of the normal guinea-pig acts but feebly on the spermatozoa of this species, which, under its influence, remain motile for

[1] *Deutsche med. Wchnschr.*, Leipzig, 1900, S. 61.
[2] *Ann. de l'Inst. Pasteur*, Paris, 1900, t. XIV, p. 369.
[3] *Ibid.*, p. 577.

several hours. When, however, guinea-pigs have received one or several injections of the spermatozoa of their own species, their serum and peritoneal lymph become distinctly toxic and render the spermatozoa motionless in a few minutes. In male guinea-pigs so prepared the serum acquires this toxic property not only for the spermatozoa of other male guinea-pigs, but likewise for those of the individual itself which furnishes the serum. This latter, then, becomes distinctly autospermotoxic.

If the spermotoxin were diffused in the plasma and other fluids of the guinea-pig which furnishes it, it ought to render motionless the spermatozoa contained in the genital organs. Experiment demonstrates, however, that this is not the case. If the male organs be removed from a guinea-pig whose serum is very autospermotoxic *in vitro*, we find, especially in the epididymis, a mass of very virile spermatozoa which for a long time retain their motility in physiological salt
[109] solution. The macrocytase, then, has not reached the spermatozoa in the living animal; this is because it is not found in the plasmas. Let us inject into a guinea-pig, whose serum is strongly autospermotoxic, one portion of sperm into the subcutaneous tissue and another portion into the peritoneal cavity. In the first site a soft oedema, filled with transuded fluid, in which the very active spermatozoa retain their motility for a couple of hours, is produced. In the peritoneal fluid the same spermatozoa become motionless in a few minutes. This great difference is explained by the fact that, under the skin, there are no, or almost no pre-existing leucocytes, whilst in the peritoneal fluid they are abundant. The phagocytes injured by the introduction of sperm into the peritoneal cavity, abandon a portion of their macrocytase, sufficient to render the spermatozoa motionless. But when Metalnikoff injected physiological salt solution into the peritoneal cavity of his autospermotoxic guinea-pigs, and then, on the following day, a quantity of sperm, the spermatozoa continued very active for more than an hour. In this case phagolysis is very transitory and insignificant; it is soon followed by a great afflux of leucocytes which bring about a rapid ingestion of the spermatozoa. Many of these elements are devoured in a living state; for even when their body is enclosed in the macrophage, their tail, left outside, continues to move very actively.

All these experiments demonstrate that in the normal state the macrocytase remains within the phagocytes and only escapes during phagolysis, or at the moment when the blood, after it has been with-

drawn from the organism, coagulates. Is it the same for the fixative? It is easy to prove that this soluble ferment circulates in the plasmas of the living organism. We have already said that the spermatozoa of a guinea-pig whose serum is very autospermotoxic, remain alive for some time in the physiological salt solution. But if we introduce them, *in vitro*, into the serum of a normal guinea-pig they remain motile but a short time (some 10—20 minutes), whilst the spermatozoa of a normal guinea-pig will live in the same serum for several hours. This difference is explained by the fact that the spermatozoa of the autospermotoxic guinea-pig, although very active, have absorbed the fixative during the life of the animal. This fixative is, as we have stated, found in the body fluids and has been able to penetrate to the male organs. Here the spermatozoa become charged with the fixative and, once transported into the serum of the normal [110] guinea-pig, rich in macrocytase, they lose their movements very quickly. At the same time the spermatozoa used for control, not having absorbed any fixative, are able to live for a long time in the same serum.

As the macrocytase remains fixed to the phagocytes there can be no doubt as to its origin; it is elaborated by these cells. Whence however comes the fixative which is free in the body fluids and which is precisely the substance that is developed in so large a quantity in the treated animals? The exact solution of this question is not easy; nevertheless there are many facts which indicate that this fixative is also of phagocytic origin. We know already that the serums of normal animals contain only small quantities or sometimes, perhaps, none of the fixative. This fixative only appears abundantly as the result of the resorption of the corresponding elements, red corpuscles or spermatozoa. This resorption, as we have said, is almost exclusively the work of the macrophages. It is just in those cases where the red corpuscles, injected into the peritoneal cavity of an animal of the same species, pass directly into the lymph, without being injured or, save exceptionally, ingested by the phagocytes, that the fixative is not formed. When the red blood corpuscles of the goose, introduced with defibrinated blood below the skin of a guinea-pig, undergo there a partial solution in the fluid of the exudation, and where the phagocytosis is more limited than in the peritoneal cavity, the production of fixative is small. When the injection of the same goose's blood is made into the peritoneal cavity of a guinea-pig and is followed by complete phagocytosis, the fixative is produced in greater abundance. There

exists, then, in all these cases a constant relation between the degree of phagocytosis and the amount of the fixative produced. As this fixative facilitates the access of the cytase to the cells and as the resorption of these elements takes place specially in the macrophages, we are bound to come to the conclusion that the fixative is a second phagocytic ferment which is produced in abundance during the process of intracellular digestion. Only, instead of remaining in the substance of the phagocytes, this fixative is in part thrown out from these elements. It passes into the plasma of the blood and into the other fluids and ends by disappearing from the organism, probably being eliminated by the excretory channels.

In the Invertebrata, where, as we have seen, the alien red blood corpuscles are also digested within the phagocytes, we have never been able to demonstrate any haemolytic property of the blood fluid, even after repeated injections of blood. We must conclude from this
[111] that in these animals the quantity of fixative is merely sufficient to bring about the solution of the red corpuscles which are within the phagocytes. In the case of fishes and higher animals (we may recall the example of the red corpuscles of the guinea-pig when resorbed into the organism of the gold-fish) the production of the fixative is much more abundant, and this ferment can be easily demonstrated by its action *in vitro*.

This over-production of a ferment which acts in the phagocytic resorption, finds its analogue in the passage of certain digestive ferments, such as amylase and pepsin in man and the dog, into the blood and urine, as mentioned in the preceding chapter.

One of the best arguments in favour of the thesis here developed, has been furnished to us by the analysis of the phenomena observed in connection with the autospermotoxic serums of the guinea-pig. This idea of autotoxins was originally put forward by Ehrlich in his memoirs, published in conjunction with Morgenroth and already repeatedly cited. Ehrlich asked himself whether the organism which resorbs, not red corpuscles of an alien species, but red corpuscles of its own species, would also be capable of developing haemolytic substances. With this object he injected blood obtained from goats into these same goats or into other individuals of the same species. He and Morgenroth[1] were, under these conditions, able to obtain isotoxic serums, that is to say serums which dissolve the red corpuscles of the goat, coming from other individuals than those which had been

[1] *Berl. klin. Wchnschr.*, 1900, S. 453.

treated by the blood and which furnished the serum. In order to obtain this result, however, they had to inject, not unaltered blood but blood mixed with water. The red corpuscles of the unaltered blood pass readily into the circulation of the animal of the same species, without being attacked by the phagocytes. Now, we know from the experiments of Bordet that the stromas of the red corpuscles suffice for the production of the fixative, whilst the haemoglobin does not incite to the development of this ferment by the organism. As the stromas, injected with a mixture of blood and water, must be devoured by the macrophages, we can readily understand that these phagocytes may serve for the elaboration of the fixative.

The resorption of the red corpuscles and that of spermatozoa which [112] we have presented as examples, may serve as types for the resorption phenomena of formed elements in general. When other species of cells are introduced into the organism, the resulting process always reveals the same character: inflammatory reaction with preponderant intervention of the macrophages; intraphagocytic digestion of the introduced elements; excessive production and excretion of the fixatives. Whilst the macrocytase is always the same in the same species of animal, the fixatives are different and specific. In addition to the haemofixatives and spermofixatives already described, we may obtain, as the result of the injection of the corresponding cells, leucofixatives, nephrofixatives, hepatofixatives, trichofixatives, etc. It does not enter into our programme to treat the subject here[1]. We wish simply to insist on those aspects of the resorption of cells which are closely connected with the problem of Immunity. In the next chapter we must, however, recur to certain features of the phenomena of resorption.

[1] We have given a sketch of the actual state of this question of cell poisons or cytotoxins in the *Revue générale des sciences pures et appliquées*, 1901, p. 1.

[113]

CHAPTER V

RESORPTION OF ALBUMINOID FLUIDS.

Resorption of albuminoid substances.—The precipitins of blood serum which appear as a result of the absorption of serums and of milk.—Absorption of gelatine.—Leucocytic origin of the ferment which digests gelatine.—Antienzymes.—Anti-rennet.—The anticytotoxins.—Antihaemotoxic serums.—Their two constituent parts: anticytase and antifixative.—Action of anticytase.—The antispermotoxins.—Origin of anticytotoxins.—Ehrlich's theory on this question.—Origin of antihaemotoxin.—Origin of antispermotoxin.—Production of this antibody by castrated males.—The antispermofixative produced when the spermatozoa are excluded.—Distribution of spermotoxin and antispermotoxin in the organism.

We stated at the beginning of the last chapter that various fluid substances of very complicated chemical composition may be absorbed by the tissues and utilised by the organism without requiring to be modified by the digestive juices of the intestinal canal. We must now describe, exactly, the phenomena observed in these cases and endeavour to establish the mechanism of the absorption of fluids in the living organism.

We have already cited the examples of blood serum, milk, and white of egg, all of which are readily utilised by the organism which receives them directly into the peritoneal cavity or below the skin. The proof that these substances are modified—digested by the tissues, is furnished by the observation that their injection necessarily brings about appreciable changes in the properties of the blood. Th. Tchistovitch[1], in a research carried out in the Pasteur Institute, was the first to demonstrate that the resorption of the blood serums of the eel and horse by the organism of the rabbit, excites in the blood of the latter animal the production of specific precipitates. The blood serum of rabbits that have been vaccinated against the toxic eel's serum gives a precipitate with eel's serum; the serum of rabbits treated with horse's blood gives a similar precipitate with
[114] horse's serum, etc. This property has since been confirmed and

[1] *Ann. de l'Inst. Pasteur*, Paris, 1899, t. XIII, p. 413.

studied by several observers, who have made use of it for the recognition of human blood in medico-legal investigations[1].

Bordet[2] has made the discovery that intraperitoneal injections of the milk of cows into rabbits provokes in the blood serum of the latter the property of giving a specific precipitate with cow's milk only. This precipitation bears a great resemblance to the coagulation of casein; which, however, does not justify us in identifying the precipitating substance with rennet. This fact has been confirmed for several other species of milk, and Schütze[3], in an investigation carried on in the Berlin Institute, essayed to apply it to the differentiation of the various kinds of milk. In the same order of ideas, researches have been made on the artificial precipitins that develop in the blood as the result of injection of white of egg and other albuminoids[4]. Leclainche and Vallée[5] have prepared animals in such a fashion that their serum produces a precipitate with urinary albumen. The biological precipitin reactions are more sensitive than any of the chemical reagents properly so called. These specific substances in the serums must be looked upon as belonging to the group of soluble ferments, approximating to the fixatives rather than to the cytases, since they are unaltered by being heated to 56° C. Their action gradually declines after passing 60° C. but is only destroyed at a temperature beyond 70° C.

An analogous soluble ferment has been discovered in the blood serum of animals treated with injections of gelatine. We owe to Delezenne, who has studied this question in his laboratory at the Pasteur Institute, the most important and most complete data on the resorption of gelatine. The blood serum of normal animals possesses only a very feeble power, sometimes even none, of liquefying gelatine. When however this substance is injected several times, the serum, as is the rule for the formed elements and quite a series of fluid substances, acquires a much more pronounced activity. The gelatine, [115] without giving any precipitate, is simply dissolved and will no longer

[1] Deutsch, *Compt. rend. XIII congrès internat. de Méd. de Paris*, and *Centralbl. f. Bacteriol. u. Parasitenk.*, I^te^ Abt., Jena, 1901, t. XXIX, S. 661; Uhlenhuth, *Deutsche med. Wchnschr.*, Leipzig, 1901, S. 82; Wassermann u. Schütze, *Berl. klin. Wchnschr.*, 1901, S. 187; [Nuttall and Dinkelspiel, *Journ. of Hyg.*, Cambridge, 1901, Vol. I, p. 367; Nuttall, *Brit. Med. Journ.*, London, 1902, I, p. 825].

[2] *Ann. de l'Inst. Pasteur*, Paris, 1899, t. XIII, p. 240.

[3] *Ztschr. f. Hyg.*, Leipzig, 1901, Bd. XXXVI, S. 5.

[4] [Myers, *Lancet*, London, 1900, II, p. 98, and *Centralbl. f. Bakteriol. u. Parasitenk.*, I^te^ Abt., Jena, 1900, Bd. XXVIII, S. 237.]

[5] *Compt. rend. Soc. de biol.*, Paris, 1901, p. 51.

solidify when it is cooled. The ferment of the serum that produces this effect resembles the precipitins in that it withstands the action of a temperature of 56° C. and is only destroyed beyond 60° C. Like the trypsins it acts in a weakly alkaline, neutral, or weakly acid medium; but digestion takes place best in a slightly alkaline medium.

The question of especial interest to us is that of the origin of this ferment which digests gelatine. If several c. c. of a 10 % solution of this substance be injected into the peritoneal cavity of a laboratory animal, there is provoked with certainty, within a few hours, a marked leucocytosis of the peritoneal fluid. A considerable afflux of leucocytes, amongst which the microphages are even more numerous than the macrophages, takes place. When to a hanging drop of such an exudation a trace of Ehrlich's neutral red solution is added, there appears almost at once an intense coloration of the numerous droplets inside the two kinds of leucocytes. It is, therefore, manifest that the gelatine excites a powerful positive chemiotaxis of the mobile phagocytes and that it is absorbed by these cells. This experiment demonstrates that the phagocytes can not only ingest solid bodies, such as the various formed elements, coloured granules, etc., but that they are also capable of absorbing fluid substances introduced into the tissues or cavities of the organism.

The data brought forward by Delezenne demonstrate very clearly the part played by the mobile phagocytes in the digestion of gelatine. He obtained his best results in the dog. We know that it is easy in this animal to provoke an aseptic exudation, very rich in leucocytes. This exudation when deprived of its serum and washed with physiological salt solution gives a solution which exerts a feeble digestive action on gelatine. If the exudation be produced in a dog that has previously received several injections of this substance, we obtain leucocytes whose extract, obtained by the same method, will digest gelatine much more actively. The digestive power of the leucocytes of the treated dog is sometimes five times greater than that of the leucocytes of the normal dog. Here, then, we undoubtedly have an acquired digestive power which reveals a great reinforcement of the phagocytic activity.

[116] In the prepared dogs the leucocytes have a much greater digestive action on gelatine than has the blood serum of the same animals, a fact which indicates that the source of the soluble ferment must be sought for in the phagocytes themselves. The results of these researches are of great service to us in the study of immunity properly so called.

For some time past attempts have been made to show that the soluble ferments, diastases, or enzymes, are closely allied to albuminoid substances. Nencki and Mme Sieber[1] support this view by their recent researches on the chemical composition of pepsin. In all the above cases there is this in common between the two categories of substances, their absorption by the organism is followed by the appearance in the blood of antagonistic ferments. Just as after the injection of milk, white of egg, serums, etc. into the cavities or tissues, specific precipitins are produced, so the injection of certain enzymes provokes the formation in the organism of antienzymes or antidiastases.

It has been known for some time that the blood serum of many animals prevents the action of certain enzymes. Thus Röden has shown that normal horse's serum retards or even completely prevents the coagulation of milk by rennet. It has often been observed, too, that normal serums hinder, more or less, the digestion of albuminoids by trypsin. It is only quite recently, however, that we have begun to prepare antienzymes by the injection into animals of corresponding enzymes. Thus, Hildebrand[2] has succeeded in obtaining an antiemulsin in the serum of rabbits, into which he had injected several separate doses of emulsin. Fermi and Pernossi[3] have prepared an antitrypsin, and von Dungern[4] has obtained an antidiastase against the proteolytic enzymes of some bacteria. But of all the antienzymes, the one that has been best studied up to the present is indisputably antirennet, obtained independently by Morgenroth[5] and Briot[6]. The former of these investigators treated goats with increasing quantities [117]
of rennet and was able to assure himself, by comparative detailed researches, of the appearance and increase in quantity of antirennet in the blood serum. The goat which gave the best result ceasing to develop antirennet it was impossible to make the antirennet potency go beyond a certain point.

Briot also obtained antirennet in rabbits into which he had injected fluid rennet on several occasions. He was able to convince himself that the antirennet of horse's serum is a non-dialysable

[1] *Zeitschr. f. physiol. Chem.*, Strassburg, 1901, Bd. xxxii, S. 291.

[2] *Virchow's Archiv*, Berlin, 1893, Bd. cxxxi, S. 32.

[3] *Zeitschr. f. Hyg.*, Leipzig, 1894, Bd. xviii, S. 83.

[4] *München. med. Wchnschr.*, 1898, 15 August.

[5] *Centralbl. f. Bakteriol. u. Parasitenk.*, I^te^ Abt., Jena, 1899, Bd. xxvi, S. 349 and 1900, Bd. xxvii, S. 721.

[6] "Étude sur la présure et l'antiprésure." Sceaux, 1900. (*Thèse d. l. Faculté d. Sc. de Paris*, no. 4.)

substance which is precipitated by alcohol and certain salts. Like the precipitins and the diastase which digests gelatine, antirennet resists a temperature of 55°–56° C.; even heating to 58° C. has no effect on the antirennet serum. At 60° C. however, the heat begins to exert an injurious effect, and after three hours at 62° C. the serum has lost all power to prevent the coagulation of the casein by antirennet. Morgenroth and Briot both state that the antirennet neutralises the rennet by a direct action.

The cell poisons, or cytotoxins, of animal origin which were treated in the preceding chapter, likewise set up the production of special anti-bodies, or anticytotoxins. The consideration of these latter has a very special interest for those who study the question of immunity from a general point of view. The first discovery of these anticytotoxins was made in connection with the study of the toxic power of the blood serum of eels. Camus and Gley[1] and, independently of them, H. Kossel[2] demonstrated that animals when treated with increasing doses of eel's serum acquire an antitoxic property which protects their corpuscles against the haemolytic action of ichthyotoxin, or the toxic substance of the blood of eels. Th. Tchistovitch[3] has not only confirmed this discovery, but has added to it new and interesting data.

When antitoxic serum is mixed *in vitro* with red blood corpuscles of the species which furnished the serum and there is added to it some haemolytic eel's serum, it will be found that the red corpuscles remain quite unaltered. In the control tubes, however, in which the antitoxic serum is replaced by normal serum of the same species, the red corpuscles are very readily dissolved under the toxic influence of
[118] the eel's serum. In animals (rabbits) that are treated with this latter fluid, there is established not only an antitoxic power of the blood, but the red corpuscles acquire a resisting power more or less pronounced against the ichthyotoxin of eel's serum. When the red corpuscles are separated from the serum of rabbits (treated with eel's serum) and some ichthyotoxin is added to them, solution very often does not take place at all. According to the experiments of Tchistovitch there is no direct relation between this acquired resistance and the antitoxic power of the blood. Sometimes even a kind of antagonism is observed between the two properties; that is to say, the red corpuscles of a rabbit whose serum is very antitoxic may

[1] *Arch. internat. de Pharmacodyn.*, Bruxelles et Paris, 1898, t. III and IV.
[2] *Berl. klin. Wchnschr.*, 1898, S. 152.
[3] *Ann. de l'Inst. Pasteur*, Paris, 1899, t. XIII, p. 406.

be extremely sensitive to the poison of the eel whilst the converse may also hold good [cf. *infra* p. 120].

The toxic action of the eel's serum upon the red corpuscles of a great number of Vertebrates is a natural property which demands no previous treatment of the eel. It is the antitoxic power, directed against the ichthyotoxin, which is developed only as a result of the preparation of the animals by the administration of increasing doses of eel's serum. Nevertheless we also find natural antitoxins present in the blood of man or animals that have not been treated and which act against the cell poisons, cytotoxins, so widely distributed in the blood of a large number of species of animals.

Besredka[1] has demonstrated that the blood serum of Man and many Vertebrates contains a substance which prevents the solution of red corpuscles under the influence of blood serums of a different species. To reveal the presence of these antitoxins it is useful to heat the serums to 56° C. and then to add to them red corpuscles of the same species and some haemolytic serum of a different species. Under these conditions the solution of the red corpuscles does not take place, whilst their mixture with haemolytic serum alone, inevitably provokes haemolysis.

Along with these natural antihaemolysins there exist a number of artificial antihaemolysins or antihaemotoxins. Jules Bordet[2] was the first to draw attention to this important subject. He first obtained these antihaemolysins by injecting blood serum of the fowl, which possesses a very great haemolytic power on the red corpuscles of the rabbit, into individuals of this latter species. After some injections, [119]
the serum of these treated rabbits was found to be antihaemotoxic against the fowl's serum. Later[3], Bordet obtained a serum against an artificial haemotoxin. The serum of the guinea-pig is innocuous to the red corpuscles of the rabbit. But when rabbit's blood was injected several times into guinea-pigs the serum of the latter became very solvent for the red corpuscles of the rabbit. To prevent this action it is sufficient to inject the haemotoxin of treated guinea-pigs several times into rabbits. The serum of these rabbits becomes antihaemotoxic and protects the red corpuscles of the rabbit against the solvent action of guinea-pig's serum.

In the normal haemolytic serums, such as the serums of the eel and

[1] *Ann. de l'Inst. Pasteur*, Paris, 1901, t. xv, p. 785.
[2] *Ibid.* 1899, t. xiii, p. 285.
[3] *Ibid.*, Paris, 1900, t. xiv, p. 270.

fowl, the presence of two substances which act by combining could not be demonstrated. On the other hand, in the serums that were obtained as a result of the treatment of animals by the injection of blood from a different species, it was easy to demonstrate, as we have shown in the preceding chapter, the presence of two constituent substances which are: the macrocytase (alexine, complement) and the fixative (amboceptor of Ehrlich, sensibilising substance of Bordet). For this reason the study of the antihaemotoxins obtained against artificial haemotoxins is endowed with special interest. As the solution of the red corpuscles, in this case, can be prevented either by an antitoxic action directed against the cytase, or by a neutralisation of the fixative (for the concurrence of these two substances is indispensable in order that the solution may take place), Bordet asked whether the antitoxic serum, obtained by him in rabbits, is anticytatic or antifixative, or whether it contains both properties. Before resolving this problem it was necessary to establish some of the essential characters of artificial antihaemotoxic serums. The principal one amongst them is the resistance of these antihaemotoxins to a temperature of 55—60° C.; even when heated to 70° C. the antihaemotoxins retain, at least in part, their fundamental property. In this respect these substances differ from the cytases and approach the precipitins, fixatives and agglutinins.

The very exact experiments carried out by Bordet have demonstrated that in the serum of rabbits, treated with the specific
[120] haemotoxic serum of guinea-pigs, two substances, an anticytase and an antifixative, are found in combination. The former of these antitoxins is found in abundance, but the amount of antifixative is very small. Bordet was led to this result in the following way. To prevent the solution of the red corpuscles of the rabbit in the haemotoxic serum of the guinea-pig, it was necessary for him to add a considerable dose (10 to 20 times) of the antitoxic serum. When, however, he heated the latter to 55° C. the quantity of this serum necessary to prevent haemolysis could be reduced very considerably. In place of its being necessary to add to the haemotoxic serum 10 or 20 volumes of antitoxic serum, it was sufficient to add three or sometimes only two volumes of this heated serum. As we know already, heating to 55° C. destroys the macrocytase which should be found in the antitoxic blood of the rabbit. This cytase by itself is incapable of dissolving the red corpuscles of the same species; but when it is added to the fixative of the haemotoxic serum of the

guinea-pig the macrocytase of the rabbit's serum dissolves them very readily. Hence the conclusion that in the haemotoxic serum of the guinea-pig there must be present a quantity of fixative sufficient to allow of the solution of the red corpuscles by the macrocytase of the rabbit's serum. This antitoxic serum, therefore, which only prevents the haemolysis on the condition of being added in comparatively large quantity, contains very little antifixative. When, by heating this serum to 55° C. we destroy the rabbit's macrocytase, the mixture of antitoxic serum of the rabbit and haemotoxic serum of the guinea-pig, which ordinarily dissolves the red corpuscles of the rabbit, now leaves them intact. The reason is that the free fixative contained in this mixture does not find any available macrocytase: that of the rabbit being destroyed by the heating, and that of the guinea-pig neutralised by the antitoxic serum. The experiment I have just described proves that this antitoxic serum contains specific anticytase. This anticytase is capable of neutralising the guinea-pig's macrocytase, but is altogether powerless against that of the rabbit. This last circumstance allows us to investigate whether the antitoxic serum of the rabbit contains, in addition to anticytase, a specific antifixative. Bordet prepared a mixture of antitoxic serum of the rabbit, heated to 55° C., with haemotoxic serum of the guinea-pig, also heated to 55° C. In this mixture the two macrocytases (that of rabbit and that of guinea-pig) have been destroyed by heat, but the antitoxins of the rabbit's serum and the fixative of the haemotoxic serum have remained intact. This mixture owing to its want of macrocytases was [121] incapable of dissolving the red corpuscles of the rabbit. By adding to it some fresh unheated serum from a normal rabbit the rabbit's macrocytase was introduced. As the latter could not be neutralised by the anticytase of the antitoxic serum and was incapable, by itself, of dissolving the red corpuscles of the rabbit, it was unable to produce haemolysis except on the condition that there is in the mixture a sufficient quantity of unneutralised free specific fixative. As a matter of fact, the red corpuscles of the rabbit are not dissolved in the mixture described; this proves that the fixative had become inactive in consequence of the presence of an antifixative in the antitoxic serum of the rabbit. I need not enter into further details of Bordet's experiments, which have fully demonstrated the fact that in the antitoxic serum of his rabbits there were really two antitoxins; an anticytase abundant in quantity, and an antifixative present in much smaller amount.

Ehrlich and Morgenroth[1] quite independently of Bordet have shown that an antihaemotoxic serum is very rich in anticytase. After making a number of injections of normal horse's serum (very rich in cytase) into a goat, they obtained in the blood serum of the latter an anticytase very active against the cytase of the horse. This antitoxic serum of the goat, as might be anticipated, contains no antifixative, the horse's serum that served for the injections coming from normal horses which contained no, or very little, fixative. Even in another case, where these investigators[2] injected a dog with sheep's serum very rich in fixative specific for the red corpuscles of the dog, they did not succeed in obtaining any antifixative. These observations in no way diminish the value of the discovery of the antifixative by Bordet, though they demonstrate that this antitoxin cannot, in certain cases, be found in the serum. Ehrlich and Morgenroth themselves throw out, in this connection, the suggestion that in these cases the antifixative remains linked to the cell which produces it, without being thrown off into the blood.

The very precise data that we have just summarised do not seem to agree with the statements of certain other investigators. Thus
[122] Schütze[3], from his researches on the antihaemotoxic serum of guinea-pigs, directed against the rabbit's haemotoxin, has arrived at the conclusion that in the former an antifixative only is produced. As he merely injected into his guinea-pigs haemotoxic rabbit's serum that had been heated to 60° C. and consequently deprived of the macrocytase, he concluded that in this serum there remained only the specific fixative capable of provoking the formation of an antitoxin. This must consequently be an antifixative. Paul Müller[4] came to a similar conclusion, after injecting rabbits with the heated haemotoxic serum of fowls. These injections caused the formation in the rabbit's serum of an antitoxin that Müller regarded as an antifixative.

Ehrlich and Morgenroth[5] objected to this interpretation, taking their stand on experiments made with the serums of normal animals. They were able to show that these serums, when injected in the fresh state or after being heated to 60° C., caused the production of a corresponding antihaemotoxin which is nothing but an anticytase. When

[1] *Berl. klin. Wchnschr.*, 1900, S. 684. Ehrlich, "Croonian Lecture," *Proc. Roy. Soc. London*, 1900, Vol. LXVI, p. 424.

[2] *Berl. klin. Wchnschr.*, 1901, S. 570.

[3] *Deutsche med. Wchnschr.*, Leipzig, 1900, S. 431.

[4] *Centralbl. f. Bakteriol. u. Parasitenk.*, I^te^ Abt., Jena, 1901, Bd. XXIX, S. 175.

[5] *Berl. klin. Wchnschr.*, 1901, S. 251.

Schütze and Paul Müller concluded that by heating the serums they had entirely deprived them of cytase elements they did not take into account the possibility of the cytases being transformed, under the influence of heat, into other bodies unable to produce haemolysis, but quite capable of provoking the formation of anticytases. Ehrlich and Morgenroth give to these new bodies, derived from cytases under the influence of temperatures between 55°—60° C., the name of *complementoids*; and these complementoids appear in the experiments of Schütze and Müller to have caused the production of antitoxins—anticytases.

In all the investigations just summarised the anticytases have been obtained by the injection into animals of various blood serums, fresh or heated. Wassermann[1] has discovered another method of arriving at the same result. He injected into guinea-pigs the leucocytes of rabbits, carefully deprived of all traces of serum. After some time the blood serum of guinea-pigs thus treated became weakly but distinctly anticytatic. From this experiment Wassermann draws the conclusion that, as has been often affirmed by several observers, the leucocytes really contain cytases.

How do the anticytases act upon the cytases? On this point all [123] observers who have studied this question have but one answer, the action of the anticytases is direct. Bordet thinks that the two substances combine so intimately that they cannot be again separated by heat. We know that the cytases are very sensitive to heat and that their haemolytic property is destroyed at 55° C. The anticytases, on the other hand, as already noted, are much more resistant to the action of heat. Bordet has prepared mixtures of haemolytic cytase serum and of antihaemolytic serum, neutral mixtures, that is to say, inactive for red corpuscles or with a very feeble action upon red corpuscles that have been sensibilised by the specific fixative. These mixtures no longer exhibit antihaemotoxic properties or they exercise this power in a very feeble degree. If in these mixtures the cytases remain uncombined alongside the anticytases, it is to be expected that heating them to 55° C. will restore the antihaemotoxic function of the anticytases; the cytases being destroyed at 55° C. there will remain in the mixtures only active anticytase. The experiments made on this point have demonstrated that the heating of these mixtures does not restore the antihaemotoxic action, that is to say, the anticytase is definitely combined with the cytase.

[1] *Ztschr. f. Hyg.*, Leipzig, 1901, Bd. xxxvii, S. 190.

Ehrlich and Morgenroth have satisfied themselves that their antihaemotoxin exerts no influence, either upon the red corpuscles or upon the fixative, and is only capable of preventing the action of the cytase. They introduced red corpuscles of the rabbit into a mixture of goat's serum, heated to 56° C. and thus only retaining its fixative, and anticytase serum. The fluid bathing the red corpuscles was then removed by centrifugalisation and the corpuscles were mixed with normal haemolytic horse's serum. Solution of the red corpuscles took place at once as the anticytase had been completely removed during centrifugalisation, being combined with neither the red corpuscles nor the fixative.

These investigators have obtained various anticytases by injecting serum of various species of animals into other mammals. They observed, however, that injections of the serum of an allied species did not bring about the formation of anticytases. Thus the injection of goat's serum into sheep, or of that of sheep into goats, never produced anticytase serum.

In addition to antihaemotoxic serums several other analogous
[124] anticytotoxic serums have now been obtained. Thus Delezenne has prepared serums which prevent the action of neurotoxin and of the cell poison which destroys the liver cells. We[1] have been able to obtain a rabbit's serum which prevents the spermatozoa of this rodent being rendered motionless by the specific spermotoxin of the guinea-pig. More recently Metalnikoff[2], working in my laboratory, has prepared another antispermotoxic serum which prevents the specific spermotoxin of the rabbit from arresting the movement of the guinea-pig's spermatozoa.

As the history of these antispermotoxins presents certain interesting general features we may with advantage, perhaps, dwell on some of their characters. The two antispermotoxins mentioned above are distinguished by certain peculiarities. When Metalnikoff set to work to inject rabbit's spermotoxin into guinea-pigs, he thought that he had an easy task before him and that after a few injections the guinea-pig's serum would become antispermotoxic. This, however, was not the case. The serum from these animals when mixed with spermotoxic serum was powerless to prevent the immobilisation of the spermatozoa of the guinea-pig. It was only when he heated the serum of his treated guinea-pigs to 56° C. that the antispermotoxic power appeared with

[1] *Ann. de l'Inst. Pasteur*, Paris, 1900, t. XIV, p. 5.
[2] *Ibid.*, p. 583.

the greatest distinctness. The inefficacy of the unheated serum must therefore depend on the toxic action of the guinea-pig's macrocytase, because it is this substance alone that can have been destroyed by the heating process. Now, in order that this macrocytase may act, the presence of the fixative is necessary, which leads us to the conclusion that the serum of the guinea-pigs injected by Metalnikoff contained no antifixative. This hypothesis was fully confirmed by experiment. Metalnikoff introduced a drop of guinea-pig's serum into a mixture of antispermotoxic serum, heated to 56° C., with spermotoxic serum. The spermatozoa continued their movements in normal fashion. But when afterwards he added a few drops of unheated serum from a normal guinea-pig the motions of the spermatozoa were arrested almost instantaneously. Consequently there was present in the mixture rabbit's macrocytase which had been neutralised by the anticytase of the prepared guinea-pig's serum and for that reason the spermatozoa remained motile. But in the same mixture we had also the specific [125]
fixative, coming from the rabbit's spermotoxic serum, which remained free and not neutralised. The motile spermatozoa had become impregnated with this fixative and a little guinea-pig's macrocytase (against which the anticytase was powerless) was sufficient to make them suddenly cease their movements.

There is no doubt, then, that the serum of guinea-pigs that have been treated with spermotoxin contains anticytase only and no, or almost no, antifixative. Such is not the case with the antispermotoxin obtained by us in rabbits that were treated with spermotoxic toxin of guinea-pigs. Several consecutive injections were sufficient to render the serum of the rabbits so treated capable of preventing the action of the spermotoxic serum of the guinea-pig on the motility of the rabbit's spermatozoa. In the mixture of antispermotoxic serum and spermotoxic serum these spermatozoa continue to move for a considerable time, whilst in the control mixture prepared with normal rabbit's serum and spermotoxic serum they become motionless at the end of a few minutes. To obtain this marked effect it was not necessary to heat the antispermotoxic serum as in Metalnikoff's case. Indeed I have performed almost all my experiments with fresh serums, unheated. As the rabbit's serum contains macrocytase capable of rendering the spermatozoa, sensibilised by the fixative, motionless and as this macrocytase cannot be neutralised by the anticytase that is active against the guinea-pig's macrocytase, the fact I have just pointed out indicates that the antispermotoxic serum of my rabbits

contains antifixative. The difference between the antispermotoxic serum obtained by Metalnikoff and that prepared by me is similar to that observed between the antihaemotoxic serums. Some contain only anticytase but others undoubtedly contain antifixative also.

As this result appeared to me to be of far-reaching importance I felt bound to verify it by another method. I injected certain rabbits with spermotoxic serum of the guinea-pig and others with normal guinea-pig's serum. The amount of cytases being about the same in both, the strength of the serums obtained as the result of injections of normal serum and of specific serum should be the same if the antispermotoxic serums contain anticytase only. Experiment demonstrates just the contrary. The antispermotoxic serum of rabbits treated with normal guinea-pig's serum was on every occasion much
[126] less active than the serum of rabbits injected with the spermotoxic serum of prepared guinea-pigs. The former contains anticytase only, whilst the latter contains in addition antifixative. Weichhardt's[1] experiments carried out in my laboratory corroborated the conclusion I have just formulated.

Having made ourselves acquainted with the constitution of the anticytotoxins we may now pass to the question of the origin of these bodies and of analogous ferments which act in the resorption of albuminoid substances in the blood and in the tissues.

We have already mentioned that the leucocytes are charged with a soluble ferment which digests gelatine, and that in animals treated with injections of gelatine these cells elaborate a much larger amount of the ferment. Here we have evidence of a kind of education of the leucocytes to produce a greater amount of digestive ferment, in a manner quite analogous to that which has been described in Chapter III in connection with the augmentation of the pancreatic ferments in intestinal digestion. It is, then, quite permissible to look upon leucocytes, and probably phagocytes in general, as the source of the soluble ferment that digests gelatine.

Is this the case with the other substances which take an active part in the resorption of albuminoid substances in the fluids and tissues of the organism? Up to the present the origin of precipitins and antiferments, such as antirennet, has not been studied. The problem being very complex and difficult, it appears to be impossible at present to solve it. It is known indeed that the introduction of these substances into the organism provokes a reaction similar to the

[1] *Ann. de l'Inst. Pasteur*, Paris, 1901, t. xv, p. 833.

one we have described in the case of the injection of gelatine into the peritoneal cavity of guinea-pigs. Thus Morgenroth[1] observed that in his goats the subcutaneous injection of sterile rennet caused the formation of extensive infiltration at the seat of inoculation, this being accompanied by fever; we are justified in concluding from this that rennet provokes a marked leucocytic reaction. Hildebrandt[2] has demonstrated by direct experiment that rennet, when enclosed in capillary glass tubes and introduced below the skin of rabbits, induces a marked positive chemiotaxis. This led to the formation of a leucocytic plug several millimetres long. Now we know from Briot that [127] the rabbit is capable of producing antirennet. Hildebrandt has further shown that several other diastases, or hydrolytic ferments, such as sucrase and emulsin, give rise to a similar chemiotactic phenomenon. The leucocytic reaction is consequently a general phenomenon following the introduction into the tissues of substances of complex chemical composition capable of provoking the formation of antibodies. We are tempted from this fact to accept it as a law that the leucocytes are capable of producing these latter substances. Although this hypothesis may be very probable, the number of facts at our disposal is not yet sufficient to justify the statement that its truth is demonstrated.

Since it is the red corpuscles which are affected by the haemotoxins it might be asked whether it may not be that these elements defend themselves by the production of antihaemotoxins the overplus of which is thrown into the blood and fluids in general? The researches that have been made on this point relate especially to the antihaemotoxin of the blood serum of rabbits in relation to the ichthyotoxin of eel's serum.

We must therefore examine the collected evidence bearing on anticytotoxins and analogous bodies and endeavour to form some idea as to their probable origin. A large accumulation of exact data bearing on the antihaemotoxins does not afford us sufficient information as to the source of these substances.

Let us first examine the question, is it possible to attribute to the red corpuscles the function of producing the antihaemotoxins? If these elements are really the source of the antihaemotoxins it is probable that the red corpuscles of animals whose serum is antihaemotoxic will exhibit marked resistance to the toxins; thus we know that the white corpuscles which produce gelatinase digest

[1] *Centralbl. f. Bakteriol. u. Parasitenk.*, I^te^ Abt., Jena, 1899, Bd. xxvi, S. 352.

[2] *Virchow's Archiv*, Berlin, 1893, Bd. cxxxi, S. 5.

gelatine much better than does the serum of the same animals. From the experiments of Tchistovitch (*l. c. supra* p. 110) on rabbits that have been immunised against eel's ichthyotoxin, it must be accepted that the red corpuscles of these animals are often very sensitive to the action of the poison at a period when the blood serum of the same rabbits exhibits a marked antihaemotoxic power. It is not until later in the process of immunisation, when the serum loses a great part of this power, that the red corpuscles become resistant to the ichthyotoxin.

But before we abandon the hypothesis of the production of anti-haemotoxins by the red corpuscles we must see if it cannot be reconciled with the facts, by the application of Ehrlich's side-chain
[128] theory[1]. This theory was evolved with the object of explaining the production of antitoxins and their action on bacterial and vegetable toxins. Later, Ehrlich has extended it to the cytotoxins, anticyto-toxins and bactericidal substances.

According to Ehrlich the complex molecule of albuminoid substances contains, besides the central stable nucleus, a number of side-chains, or "receptors," which fulfil various accessory functions and serve especially for the nutrition of the cell. These receptors have a great affinity for the various substances necessary for the maintenance of the life of the cell. Under normal conditions these receptors seize nutritive molecules, as a leaf of *Dionaea* seizes the fly that serves it as food. Under special conditions these receptors lay hold of complex molecules of albuminoid substances, such as the various toxins. In this case the receptor, instead of combining with a molecule which supports life, fixes a molecule which poisons the cell. According to Ehrlich's theory on the constitution of toxins their molecules contain an atomic group which poisons—the *toxophore*, and another group which combines with the receptor—the *haptophore*. The toxic group of a complex poison, such as ichthyotoxin, cannot penetrate into a red corpuscle except by the help of the haptophore group and of the corresponding receptor. When a red corpuscle has absorbed a large number of molecules of ichthyotoxin, the united action of the toxo-phore groups renders life impossible and the corpuscle is dissolved. But when a red corpuscle has been touched by only a few toxic molecules, too few to compromise life, there is merely immobilisation

[1] *Klin. Jahrb.*, Jena, 1897, Bd. VI, S. 299; "Croonian Lecture," *Proc. Roy. Soc. London*, 1900, Vol. LXVI, p. 424. Ehrlich, Lazarus u. Pincus, "Leukaemie, etc." in Nothnagel's *Specielle Pathologie u. Therapie*, Wien, 1901, Bd. VIII, Schlussbetrachtungen, S. 163.

of the receptors which are combined with the haptophore groups of the ichthyotoxin. As these receptors fulfil an important function in the nutrition of the red corpuscles, the latter reproduce them in larger numbers than were originally present. We know that in the phenomena of repair an over-production of the new-formed parts often takes place and, according to Ehrlich, to this over-production the presence of antitoxins in the fluids of the body is due. The receptors, developed in excess by the red corpuscles, fill these cells, and no longer finding room therein are extruded from them and overflow into the blood and other fluids of the organism. When a fresh injec- [129]
tion of toxin makes its way to the blood it there meets with a number of free receptors, endowed with an affinity for the haptophore group of the molecule of the toxic substance. The chemical combination between the two substances takes place at once in the plasmas, a fact which prevents the haptophore group of the toxin from uniting with the receptor of the red corpuscles and so injuring these cells by introducing the toxophore group into them. According to this theory the same receptors which, in the free state in the fluids, fulfil the *antitoxic* function become in the interior of the red corpuscles the vehicles of intoxication and consequently fulfil a *philotoxic* function. This opposite *rôle* of the receptors has often been compared to a lightning-conductor; so long as the receptors are attached to the molecule of the living protoplasm they attract the toxin just as a lightning-conductor attracts the lightning when it is badly insulated.

So interpreted, it is easy to conceive that the red corpuscles of animals whose fluids are antihaemotoxic may be sensitive to the toxic action of eel's serum, as has been observed by Tchistovitch. As soon as the protective fluids have been removed from the red corpuscles of the immunised organism, the corpuscles when placed in contact with ichthyotoxin (eel's serum) attract the haptophore groups of the poison by means of their numerous receptors. These haptophores in their turn introduce the toxophore groups which dissolve the red corpuscles without the slightest difficulty. This theory does not explain the cases, which are numerous, in which the red corpuscles of rabbits that are vaccinated against eel's poison resist this poison. Camus, Gley, and Kossel, working independently, have arrived at the result that the red corpuscles of immunised rabbits, from which the serum has been carefully removed, are not dissolved when submitted to the action of ichthyotoxin, whilst the red corpuscles of untreated rabbits placed under the same conditions, undergo a rapid solution.

Tchistovitch confirming this fact has added to it the observation that the resistance of the red corpuscles of the rabbit is most often found when the serum loses its antitoxic power. If the receptors of the red corpuscles of immunised rabbits, owing to their great affinity for the haptophore group of the ichthyotoxin molecule, only attract the toxophore group of this poison, as the lightning-conductor when badly insulated attracts the lightning, the red corpuscles should
[130] never manifest resistance. To explain this contradiction we must not suppose that the red blood corpuscles which have become resistant have got rid of their receptors. In fact, if these receptors are so necessary to the nutrition of the cell that their absence has set up this extraordinary over-production which has inundated the fluids, it is evident that one cannot admit the existence of red corpuscles entirely deprived of corresponding receptors.

When examined from different points of view the hypothesis of the production of antihaemotoxin by the red corpuscles is surrounded with very great difficulties. It appears to be probable, therefore, that the source of this antitoxin must be sought for in other cell elements, and we may be allowed to recall to mind those cells which manifest a general and local reaction of the most constant kind after each injection of ichthyotoxin. Tchistovitch has observed that eel's serum when introduced into rabbits in non-fatal but immunising doses excites a marked hyperleucocytosis.

The question of the origin of anticytotoxins being so complicated, it has been necessary for its elucidation to seek an experimental method of excluding the organ in which the antibody is supposed to have its origin. As we cannot think of eliminating the red or white corpuscles, nor the greater part of the tissues and organs, there remains only one way of bringing about this result. It is the suppression of the male genital organs. We know already that the injection of semen readily excites the production of a spermotoxin, and that this spermotoxin gives rise to the development of a corresponding antispermotoxin. If it is the spermatozoa, that is to say the elements having a particular affinity for the spermotoxin, which elaborate the antitoxin we must conclude that castrated males would be incapable of producing it. With this in view we have carried out a great number of experiments which have amply proved to us that male rabbits when deprived of their sexual organs are fully as capable of developing antispermotoxin in their fluids as are control rabbits in which the male genital apparatus remains intact. Doe-rabbits,

and young, sexually immature rabbits of both sexes, also react to injections of spermotoxin by producing the corresponding antispermotoxin. The specific elements which are sensitive to the action of a cytotoxin undoubtedly are not indispensable for the development of the corresponding anticytotoxin. This result is in complete harmony with the hypothesis above put forward, that the red corpuscles cannot [131] be regarded as the source of the antihaemotoxin. In the case of antispermotoxin this fact can be rigorously established by experiment.

Here arises the following question. We have seen that the anticytotoxins are composed of two different substances: an anticytase and an antifixative. The former is an antitoxin capable of neutralising macrocytase, the soluble ferment which will attack indifferently all kinds of cell elements. It is not to be wondered at, then, that the exclusion of the spermatozoa in no way prevents the production of anticytase by an organism which receives injections of cytotoxins. These latter, as we have already said, contain cytase along with the specific fixative; the macrocytase can attack any kind of animal cell provided that it can find some fixative or any other means to penetrate into the interior of these formed elements. We have seen that the antispermotoxin, obtained by Metalnikoff in guinea-pigs, does not contain any anticytase. Amongst his animals treated with spermotoxin was a castrated male guinea-pig which also produced anticytase. There is nothing astonishing in this fact, the injected cytase must have linked itself to many other cells which were able to develop anticytase.

But the example of the antispermotoxin of the rabbits in my own experiments is very different. In order that it might manifest its action the serum of these rabbits did not need to be heated to 56° C.; it was not necessary to rid it of its own macrocytase which could have acted under the influence of the fixative, if this latter for want of antifixative had remained free in the added spermotoxin. This antifixative, then, is undoubtedly found in the serum of castrated males which have shown themselves capable of producing not only anticytase, but also antifixative. This result has been further verified by comparative experiments on castrated male rabbits, some of which received spermotoxic guinea-pig's serum whilst the others received only normal guinea-pig's serum. It has been demonstrated that the amount of cytases remains almost constant in both normal and vaccinated animals[1]. If, then, the antispermotoxins contain only

[1] Bordet, *Ann. de l'Inst. Pasteur*, Paris, 1895, t. IX, p. 499; von Dungern, *München. med. Wchnschr.*, 1900, S. 678.

[132] anticytase, the injection of specific guinea-pig's serum and that of normal guinea-pig's serum should produce the same result, that is to say the serums of castrated rabbits, when treated by these two kinds of guinea-pig's serum, should exhibit the same antispermotoxic power. Experiments have, however, proved that this is not the case. The serum of castrated rabbits that have been injected several times with normal guinea-pig's serum becomes distinctly antispermotoxic, but its power to protect the spermatozoa of the rabbit against being deprived of motility by the guinea-pig's spermotoxin is greatly inferior to that which is developed in the serum of other castrated rabbits that I injected with spermotoxic guinea-pig's serum. Of course all the other conditions of the experiment were the same for the two groups of rabbits.

Several series of facts, then, focus to this fundamental point, that the organism of an animal that has been deprived of its male sexual organs is in a condition to produce antispermofixative. Against the argument that we have drawn from the fact that the antispermotoxic serum of castrated rabbits that have been treated with spermotoxic serum acts without being heated, might be cited certain experiments made by Ehrlich and Morgenroth. The antispermotoxic action in this case, as already stated, demonstrates that the serum of prepared rabbits contains antifixative. Otherwise, had the fixative not been neutralised, it would have allowed the macrocytase of the rabbit's serum to arrest the movements of the spermatozoa. Now the two above-named observers have demonstrated[1] that the injection of different serums into animals is capable of exciting in their blood the development of anticytases. The macrocytase of castrated rabbits which, before treatment with the spermotoxin, was capable of arresting the movements of rabbits' spermatozoa acted upon by a fixative, might become inert after the injections of spermotoxic serum of guinea-pigs. To clear up this point I asked M. Weichardt[2], who has carried out work on this subject in my laboratory, to try by means of unheated serums of normal animals, to restore the activity of spermotoxin that had been mixed with antispermotoxic serum. Spermatozoa of rabbits were put into a definite mixture of spermotoxic guinea-pig's serum, heated to 56° C., and antispermotoxic serum, also heated to 56° C., obtained from castrated rabbits that had been treated with spermotoxin. The spermatozoa remained very active in this

[1] *Berl. klin. Wchnschr.*, 1901, S. 255.

[2] *Ann. de l'Inst. Pasteur*, Paris, 1901, t. xv, p. 833.

mixture which contains specific fixative (in the spermotoxic guinea- [133]
pig's serum) and antispermotoxin. To this mixture is added a little normal rabbit's or horse's serum, unheated. These serums contain cytases and would be quite capable of arresting the movements of the spermatozoa if there was found in the mixture any free fixative that would enable the macrocytase to be linked to the spermatozoa. Under these conditions the spermatozoa remain motile for a long time. The fixative, then, was no longer active; it was neutralised by the anti-fixative of the antispermotoxic serum of castrated rabbits. A control experiment was made with the same substances; but the castrated rabbits' serum that had been treated with spermotoxic serum was replaced by the serum of other castrated rabbits treated with normal guinea-pig's serum. In these latter mixtures the spermatozoa became motionless at the end of a very short time; the fixative, not being neutralised, readily allowed the rabbit's and horse's cytases to affect the spermatozoa.

It follows from all this that the antispermotoxic serum of castrated male rabbits, when treated with normal guinea-pig's serum, contains anticytase only; whilst the serum of castrated male rabbits, treated with specific and spermotoxic guinea-pig's serum, contains anticytase and antifixative. The latter, then, has been produced independently of the sensitive elements,—the spermatozoa.

Having established the fact that antispermotoxin does not come from the male organs, it was necessary to try to ascertain its true source. With this object in view we injected spermotoxic serum into young rabbits (quite capable of producing antispermotoxin) and tried to follow the fate of the spermotoxin in the organism. When spermotoxic guinea-pig's serum is injected into the peritoneal cavity of the rabbit a notable amount of spermotoxin is found in the thickened portion of the omentum made up of lymphoid tissue. But the greater portion of the poison passes into the circulation whence it goes to fix itself in various organs, especially the spleen. At the moment when the spermotoxin is found in the blood a certain quantity of this fluid was drawn off into tubes containing some drops of extract of leeches' heads. After the blood thus treated had been centrifugalised the plasma was decanted and its power of arresting the movements of spermatozoa was compared with that of serum of the same blood prepared in the usual way. From these researches it results that the plasma is always richer in spermotoxin than is the corresponding [134]
serum. Sometimes the difference in favour of the plasma is very great.

A part of the spermotoxin passes into the kidneys and the suprarenal capsules. It is probable that, as is the case with so many soluble poisons, a certain proportion of the spermotoxin may be eliminated by the uropoietic organs. A small quantity of this poison is found also in the male and female sexual glands of young non-castrated rabbits.

The search for some main centre of origin for the production of antispermotoxin has as yet led to no positive result. The power of arresting the movements of spermatozoa first appears in the blood plasma, and it is this same fluid which, later, is more antispermotoxic than is any organ. Amongst the tissues which fix spermotoxin the genital organs play not the slightest part in the production of antispermotoxin. The experiments with castrated rabbits afford sufficient proof of this. On the other hand it becomes more and more probable that the phagocytic system, disseminated in many organs, and especially the leucocytes, furnish the antispermotoxic substance. The fixation of the spermotoxin by the leucocytes of the blood, such as the cells of the omentum and of the spleen, already offers us a valuable indication. The absence of any particular organ that might have the monopoly of fixing the spermotoxin and which should later be found charged with a predominant amount of antispermotoxin also speaks in favour of the phagocytic origin of this antitoxin.

After a single intraperitoneal injection of spermotoxic guinea-pig's serum into young rabbits, the blood of the latter is distinctly spermotoxic for several days; later it becomes indifferent, but eight or ten days after the commencement of the experiment the blood begins to exhibit an antispermotoxic power. In these cases the plasma shows itself more active than the serum. When the rabbits are killed at this stage of commencing antitoxic production, it is found that an extract of the organs is not antispermotoxic or only feebly so. In all cases this power, when it exists, is more feeble than that of the blood fluid. The results obtained with extracts of organs are not constant. Sometimes the spleen possesses more antitoxic activity, whilst the liver, thymus, omentum, lymphatic glands and genital glands exhibit none of this property. In other cases the survival of the spermatozoa that are
[135] influenced by the spermotoxin has been longest in the extract of the suprarenal capsules. Sometimes the extract of the omentum exhibits the greatest antispermotoxic power. This great variability in the development of the property of protecting the spermatozoa accords well with the idea that the elements which produce antispermotoxin

are wandering cells which, under diverse influences, may be localised in very diverse points of the organism.

We must not deceive ourselves. The facts which have been collected up to the present do not allow us as yet to form a final opinion on the origin of anticytotoxins, but we are quite justified in regarding as very probable the hypothesis that the phagocytes play a most important part in the process. It is in all cases beyond doubt that the amoeboid cells which resorb the formed elements play a very important part in the resorption of fluids of very complex molecular composition.

[136]

CHAPTER VI

NATURAL IMMUNITY AGAINST PATHOGENIC MICRO-ORGANISMS

Natural immunity and the composition of the body fluids.—Cultivation of the bacteria of influenza and pleuro-pneumonia in the fluids of refractory animals.—Resistance of *Daphniae* to the Blastomycetes.—Examples of natural immunity in Insects and Mollusca.—Immunity of Fishes against the anthrax bacillus.—Immunity of frogs against anthrax, Ernst's bacillus, the bacillus of mouse septicaemia and the cholera vibrio.—Natural immunity in the cayman.—Immunity of the fowl and pigeon against anthrax and human tuberculosis.—Immunity of the dog and rat against the anthrax bacillus.—Immunity of Mammals against anthrax vaccines.—Immunity of the guinea-pig against spirilla, vibrios, and streptococci.—Natural immunity against anaerobic bacilli.—Fate of Blastomycetes and *Trypanosomae* in the refractory organism.

In the third chapter reference has been made to the frequency of cases of natural immunity against infective diseases. Examples of this immunity occur in the lower animals—the Invertebrata—and are widely met with among the Vertebrata. We have already mentioned that this natural immunity can be attributed neither to insusceptibility to microbial toxins nor to the elimination of the micro-organisms by the excretory channels. Nevertheless the pathogenic agents which have penetrated into the tissues of the refractory organism disappear, without being eliminated. To facilitate the study of their disappearance it has been necessary to pass in review the phenomena that follow the introduction of foreign bodies into the organism and to present a brief analysis of the process of resorption of cell elements in its relations to digestion. We have tried to demonstrate that resorption is nothing more than a process of digestion which, instead of going on in the intestinal canal, takes place in the tissues; that it is, indeed, an intracellular digestion exactly comparable to that which serves for the nutrition of certain of the lower animals.

A knowledge of all these facts is necessary before we can deal with the subject to which the present chapter must be devoted—the innate natural immunity of animals and man against pathogenic [137] micro-organisms. As, under natural conditions, it is the micro-organism and not its toxic products which invades the organism, it is clear that we must give the first place to the study of immunity against the micro-organism. The more so because this form of immunity is much more frequently met with than is an insusceptibility to toxins.

Since the animal organism has a very variable composition it might be concluded that the micro-organisms find in the refractory species simply a chemical medium in which they cannot live. We cannot go far in the discussion of this supposition without seeing that it may be rejected. Among the pathogenic micro-organisms some are distinguished by a great fastidiousness and sensitiveness as regards the medium in which they are placed. Such, for example, are the parasites of malaria and their allies. They live inside the red blood corpuscles of Vertebrata and appear to be extremely discriminating in regard to their requirements. All animals, even monkeys, are refractory to human malarial fevers. It might be concluded from this that here at least the immunity may be due to the fact that the chemical composition of the contents of the red corpuscles in the immune animals is different from that of the red corpuscles of man. But when we see, as was first demonstrated by Ross[1], that the malaria parasite of Laveran, having made its way into the digestive canal of certain mosquitos (*Anopheles*), there develops abundantly, it is difficult to maintain this thesis.

Among other micro-organisms of animal origin we have the *Trypanosoma*, the parasite of the terrible disease propagated by the Tsetse fly which commits such ravages amongst mammals. Man alone escapes it, exhibiting a natural immunity that nothing apparently can overcome. Are we to affirm that it is the difference in the chemical composition of the human body which assures to man his immunity against a parasite that attacks indifferently an herbivorous animal, such as the ox or rabbit, or a carnivorous animal, such as the dog? In these examples I have chosen merely those micro-organisms which it has never been possible to cultivate on any artificial nutrient

[1] *Brit. Med. Journ.*, London, 1897, II, p. 1786; 1898, I, p. 550. *Ann. de l'Inst. Pasteur*, Paris, 1899, t. XIII, p. 136.

medium and which are kept alive with very great difficulty outside the living organism.

What is to be said then of the vegetable micro-organisms which, in
[138] this respect, are much less exacting? The most important of these and the most numerous of all pathogenic micro-organisms, the Bacteria, can as a rule be cultivated without difficulty not only in the blood and fluids of animals that are susceptible or refractory to their morbific action, but also on all kinds of vegetables and artificial media: broths, fluids composed of mineral salts and of certain organic substances. It is really not possible to attribute the natural immunity of the dog and the fowl against the anthrax bacillus—so fatal to a great number of mammals, man included,—to its incapacity to feed on the fluids of these animals, when we see that this same bacillus is capable of killing lower animals, such as the cricket, and can thrive on carrots, potatoes and other vegetables.

Even when, among the bacteria, we take those that are most exacting in the choice of their food, we still find it impossible to explain natural immunity as being due to the want of power on the part of these organisms to obtain their nutriment from the juices of refractory species. The bacillus discovered by R. Pfeiffer[1] in influenza does not develop on media that are ordinarily employed in bacteriology in the cultivation of a great number of micro-organisms. It needs a special food, which is prepared for it by spreading a drop of fresh blood on the surface of agar. Pfeiffer has established the fact—confirmed by many observers—that the best species of blood to use for this purpose is that of the pigeon. We should have to believe, then, did the immunity really depend on the composition of the fluids, that the pigeon is the least refractory of all animals. Experiment has demonstrated the erroneousness of such a supposition: the pigeon is quite as refractory to Pfeiffer's bacillus as are most other species of animals.

As a second example the bacterium of bovine pleuro-pneumonia may be cited. It is the smallest of all known bacteria. The difficulties surrounding the discovery and identification of this organism were very great, and the ingenuity of Nocard and Roux[2] was required for the demonstration of its existence. Very exacting in its choice of nutritive material, it was first cultivated in the fluids of the rabbit, a species endowed with an absolute immunity against bovine pleuro-

[1] *Ztschr. f. Hyg.*, Leipzig, 1893, Bd. XIII, S. 357.
[2] *Ann. de l'Inst. Pasteur*, Paris, 1898, t. XII, p. 240.

pneumonia. It is unnecessary to multiply examples to obtain a general proof that natural immunity against micro-organisms cannot be explained by the incapacity of these pathogenic agents to live in [139] the fluids of the refractory organism.

We must, however, ascertain what takes place in resistant animals inoculated with micro-organisms. Here, again, it is preferable to begin with the lower animals of simple organisation. We have already seen that examples of natural immunity are not rare in the Invertebrata. When engaged in the study of the disease found in *Daphniae*, small crustacea so common in fresh water, I was able to show that the special Blastomycetes which cause it meet with a vigorous resistance on the part of the organism. As the *Daphniae* are small, transparent, and consequently easily observed under the microscope, I was able without difficulty to establish the main phenomena observable in these organisms. I can be the more brief in describing these phenomena of resistance as, in addition to devoting a special memoir to the *Daphnia* disease[1], I have, in my *Lectures on Inflammation* (pp. 97—103)[2], described at some length the reaction of their organism to the *Monospora*. It is nevertheless necessary that I should recall, very briefly, the mechanism by which these small crustaceans secure immunity.

The spores of the parasite—very delicate and rigid needles—are swallowed with the food. By means of their sharp points they perforate the intestine and penetrate into the body cavity, full of blood, where they find themselves exposed to the attacks of leucocytes. These leucocytes, guided by their tactile sense, gather around the foreign body, ingest it completely and destroy it. It is remarkable that the spore, which is furnished with a very resistant membrane, once in the interior of the mass of leucocytes, undergoes modifications which afford evidence of the presence in these cells of an extraordinary digestive power. The surface of the spore, from being smooth and regular, becomes pitted and sinuous, the spore breaks up into fragments and is reduced to a mass of *débris* which, in the form of brown granules, remains indefinitely in the contents of the leucocytes. From this it is evident that these phagocytes must produce a ferment which is capable of digesting the cellulose or analogous substance which forms the membrane of the spore. Unfortunately, the small size of the *Daphniae*, so useful for the

[1] *Virchow's Archiv*, Berlin, 1884, Bd. xcvi, S. 177.

[2] [English translation, pp. 83—86.]

direct observation of the phenomena of immunity, presents an insurmountable obstacle to the study of its leucocyte ferments, especially *in vitro*.

[140] The destruction of the spores of the parasite by the leucocytes secures to the *Daphnia* a real immunity. Of a hundred *Daphniae* taken in my aquarium and carefully examined under the microscope, fourteen only were found to be infected by the budding conidia of the parasite, whilst fifty-nine of the others contained the remains of spores that had been destroyed by the phagocytes. When transferred to pure water containing no new source of contagion, these *Daphniae* flourished and lived a normal life, giving birth to a numerous progeny.

The immunity of the *Daphnia*, due to the intervention of phagocytes, is an example of natural, individual immunity. It is not the specific or racial possession of these crustacea, for when the leucocytes do not seize the spore, at once, on its penetration into the body cavity, it commences to germinate and gives rise to a whole generation of budding cells. These cells, then, secrete a poison which not only repels the leucocytes, but kills and completely dissolves them. Under these conditions the *Daphnia* is disarmed; the parasites grow in the organism, deprived of its arm of defence, as in a culture tube, and the animal rapidly succumbs.

Since I first observed this struggle between the *Daphnia* and its parasite, some eighteen years ago, no other example has been found that is so easily observed and so demonstrative of the protective action of phagocytes in an animal that can be kept under observation, alive, under the microscope. Cases, however, are not wanting in the Invertebrata in which the different phases of this struggle may be observed with sufficient accuracy to warrant the conclusion that in these cases also the phenomena are analogous to those observed in the case of the *Daphniae*.

It has already been stated in Chapter III. that the larvae of the rhinoceros beetle (*Oryctes nasicornis*), although very sensitive to the cholera vibrio, are very refractory to anthrax and diphtheria. In order that we may obtain some idea of the mechanism of this immunity let us inject into the body cavity of these large white grubs a trace of anthrax culture. In the blood, drawn off the following morning, the injected bacilli are found, not in the plasma, but inside many of the leucocytes. Here there has occurred, as in the *Daphnia*, an ingestion of the parasites which have then been destroyed by the intracellular digestion of phagocytes. The process is the same, then,

as that by which the resorption of the red corpuscles of the goose takes place when they are injected into the blood of cockchafer larvae. [141] In both cases the foreign bodies are ingested and destroyed by the leucocytes of the blood; this act of resorption, however, taking a very long time.

Although the leucocytes of the larvae of the rhinoceros beetle exhibit a positive chemiotaxis for the bacillus, these same cells behave in a very different fashion in presence of the cholera vibrio. Very small quantities of this vibrio, when injected into the blood of the larvae, give them a fatal disease: the vibrios excite in the leucocytes a negative chemiotaxis and flourish without hindrance in the blood plasma. The larva is soon transformed into a culture vessel and the numerous vibrios that develop in it cause the death of the animal.

The difference in action of the two bacteria cannot be explained by any corresponding difference in their mode of life in the blood. Removed from the organism the blood plasma of the white larvae of the rhinoceros beetle is a culture medium just as favourable to the growth of the anthrax bacillus as to that of the cholera vibrio. Moreover, the former of these micro-organisms is quite capable of setting up a fatal disease in other representatives of the class of Insects. Kovalevsky[1] has discovered in the house cricket four phagocytic organs, with a great appetite for all kinds of foreign particles that may penetrate into its body. The blood of mammals, when injected below the skin of the cricket, is rapidly absorbed by the cells of the four "spleens" (for so Kovalevsky designates these phagocytic organs). The resorption of the red blood corpuscles goes on within these phagocytes owing to their power of intracellular digestion. When Kovalevsky kept crickets at a temperature of 22°—23° C. and injected them with anthrax bacilli he noted that these bacilli also were ingested by the cells of the spleens. There was, thus, no manifestation of negative chemiotaxis of these elements towards the bacillus. The ingestion of the bacilli by the phagocytes was not sufficient, however, to protect the animal. The bacilli reproduced themselves rapidly in the blood fluid; the intracellular lacunae of the spleens were full of them and the crickets quickly succumbed to the infection.

Nevertheless these crickets are quite capable of resisting certain other bacteria. Balbiani[2] has shown that they are refractory to [142]

[1] *Bull. Acad. d. sc. de St Pétersb.*, 1894, t. XIII, p. 437.

[2] *Compt. rend. Acad. d. sc.*, Paris, 1886, t. CIII, p. 952.

a great number of bacilli belonging to the group of *Bacillus subtilis*. He observed that when injected into the body of the cricket these bacilli are devoured and destroyed by the leucocytes of the blood and by the large cells of the pericardial tissue corresponding to the elements of the spleens of Kovalevsky. Whilst the crickets and other Orthoptera, which are rich in phagocytes, exhibit a real immunity against these bacilli, insects which have very few leucocytes such as butterflies, flies and Hymenoptera are found to be much more susceptible to infection by the same bacilli. In this case the direct relation between immunity and phagocytosis is very marked.

The Mollusca also furnish some interesting examples of natural immunity. Karlinsky[1] has observed that anthrax bacilli, when injected into the blood of slugs and snails, soon disappear from their bodies; these pulmonate Gasteropods are absolutely unaffected by this bacillus so formidable for many species of animals. From the rapidity of this disappearance of the bacilli it has even been concluded that it was impossible for this bacillus to live in the fluids of Mollusca. Kovalevsky (*l.c.* p. 443) has studied this question with the carefulness that characterises all his work. He confirms the fact that snails (*Helix pomatia*) resist the introduction of a large quantity of anthrax bacilli into their bodies; he notes also that these bacteria disappear from the blood. But he finds them again in the tissues of the foot, and especially in the cells which surround the pulmonary vessels. "The greater number of the bacteria are found in the cells of that part of the pulmonary region in *Helix* which adjoins the heart and kidney. All the bacteria were ingested by the cells and I easily succeeded in demonstrating this not only in sections but also in bulk" (p. 444). The snails remained in good health in spite of the presence in their phagocytes of numerous bacteria which maintained themselves there for some time. At the end of ten or twelve days and more these bacteria still presented their usual aspect; this accords well with the slowness with which intracellular digestion goes on in the majority of the Invertebrata. These bacteria were, however, no longer living, although still un-
[143] digested. Morsels of the pulmonary tissue of the snails that were injected with anthrax bacilli still gave cultures 48 hours after injection and contained bacilli capable of giving fatal anthrax to mice. Later, media seeded with similar particles remained sterile, and mice inoculated therewith continued to live. From these experiments it may

[1] *Centralbl. f. Bakteriol. u. Parasitenk.*, Jena, 1889, Bd. v, S. 5.

be accepted that bacteria, living in the blood plasma, become the prey of phagocytes which render them inoffensive and kill them. This example demonstrates once again that the organism gets rid of bacteria by the same mechanism as that which serves for the resorption of any of the formed elements. The snail reacts to bacteria as it does to the red corpuscles of the goose.

It is unnecessary to insist further on the natural immunity of the Invertebrata, and it is useless to multiply examples which always point in the same direction: to the importance of phagocytic reaction and of intracellular digestion in resorption and immunity. We must pass on to the examination of the reaction phenomena of the vertebrate organism towards pathogenic micro-organisms, following, as hitherto, the comparative method. We will commence with the study of the natural immunity of fishes as lower representatives of the great group of the Vertebrata.

It is well known that fishes are liable to infective diseases and pisciculture has often to deplore considerable losses brought about sometimes by certain of the lower Fungi (*e.g. Saprolegniae*), sometimes by Bacteria. The pathogenic microbes which produce epidemics in fishes are still little understood; but among the bacteria which kill many of the higher animals are some which cause fatal maladies in certain fishes. Thus the anthrax bacillus so virulent for many mammals is capable also, as we have seen, of producing an infection in the cricket, and may cause the death of small marine osseous fishes, the *Hippocampi*. Sabrazès and Colombot[1], who have studied this question, have demonstrated that the anthrax bacillus, which is virulent for the rabbit, when inoculated into these fishes first produces swellings at the seat of inoculation and ultimately becomes generalised throughout the body, producing a fatal septicaemia. As these experiments have given this result at a temperature of 14°—16° C., it is quite evident that the bacillus, in order to manifest its pathogenic effect, in no way needs the high temperature [144] of the mammalian body for its action.

Now among fishes there are not wanting species which resist the anthrax bacillus. Mesnil[2] has, in our laboratory, thoroughly studied the mechanism of this immunity. He has shown that several fresh-water fishes, *e.g.* the perch (*Perca fluviatilis*), the gudgeon (*Gobio fluviatilis*), and the gold-fish (*Carassius auratus*), will resist an

[1] *Ann. de l'Inst. Pasteur*, Paris, 1894, t. VIII, p. 696.
[2] *Ann. de l'Inst. Pasteur*, Paris, 1895, t. IX, p. 301.

injection of a considerable number of bacilli into the abdomen. When kept at temperatures of 15°—20° C. or even 23° C., a temperature at which the bacilli are able to develop very abundantly, these fishes destroy a large number of the bacteria in their bodies. Soon after the introduction of the bacilli into the peritoneal cavity, the numerous leucocytes accumulate around them and ingest them by the same mechanism that is observed in the Invertebrata or in the same fishes when absorbing the red blood corpuscles of alien species. In the gudgeon, at as early as six and a half hours, a very marked, nay, an almost complete phagocytosis is set up.

It is impossible to doubt the fundamental fact that the bacilli, at the moment of their ingestion, are in a perfect condition of vitality and virulence. The fluid of the peritoneal exudation, when withdrawn from the animal, is of itself incapable of preventing the development of the anthrax bacilli. The peritoneal lymph of the above-mentioned fishes is, *in vitro*, even a good culture medium for these bacilli.

When, long after the completion of the phagocytosis by the leucocytes of the peritoneal exudation, a drop of the exudation is withdrawn and kept outside the organism under suitable conditions of temperature and moisture, a number of the ingested bacilli begin to multiply and give an abundant culture. This experiment proves, indisputably, that the bacilli are devoured in the living state. If a little of the peritoneal exudation, withdrawn several (up to nine) days after the injection of the bacilli, be injected below the skin of guinea-pigs these animals die from generalised anthrax, a fact which demonstrates that the bacilli, which have been ingested alive, have retained their virulence a long time after they have been devoured by the leucocytes. But, if the peritoneal exudations that have been withdrawn at still longer periods after injection be examined, it is found that they
[145] no longer contain bacilli capable of developing in culture media or of setting up the disease in the most susceptible animal. Hence it follows that in the organism of the refractory fish, the bacteria are not destroyed by the fluids but by the phagocytes, which take a long time to bring about the complete intracellular digestion of ingested micro-organisms.

The phagocytes which assure immunity to the osseous fishes that were studied by Mesnil belong principally to the group of haemo-macrophages. These are leucocytes with abundant protoplasm which stain readily by basic aniline dyes, mononuclear cells whose

nucleus, however, is sometimes divided into lobes. It is to be noted that in the perch these are the sole representatives of the motile phagocytes, and that in this fish not only the eosinophile but every other variety of granular leucocyte is completely wanting. In the gudgeon, in addition to haemomacrophages, some microphages whose protoplasm stains faintly with acid aniline colours are met with. These facts will be useful to us when we come to study the part played by phagocytes in immunity from a general point of view.

Another class of cold-blooded animal, the Amphibia, has been much more frequently studied from the point of view of infection and immunity. The frog, an animal so convenient for many physiological and pathological researches, has been much employed for the study of immunity against pathogenic micro-organisms. Quite a literature, which has been excellently summarised in the memoir of Mesnil already cited, and to which we shall have occasion to return more than once, has been accumulated on the subject.

The immunity of frogs against the anthrax bacillus was early demonstrated and studied in Robert Koch's celebrated memoir[1] on anthrax. This observer, after injecting an emulsion of anthrax spleen into the lymph sac of the frog, recovered the bacilli from the interior of round cells which burst readily when transported into water. Koch, accepting the view then generally held, thought that the bacilli found a favourable culture medium in the contents of certain cells, but that, in spite of this, the frog was capable of manifesting a real immunity against anthrax. Gibier[2] made the [146] interesting discovery that frogs when subjected to the influence of high temperature (about 37° C.) lose their natural immunity and readily contract fatal anthrax.

Since that time the mechanism by which the organism of the frog secures immunity against the anthrax bacillus has repeatedly been studied. In a memoir which appeared in 1884[3] I insisted that the principal part played in this immunity belonged to the phagocytes which devour the injected bacteria and subject them to intracellular digestion. The round cells described by Koch are nothing but the leucocytes of the lymph sac which have seized upon the anthrax bacilli. These bacilli instead of thriving in the cell contents find there a very unfavourable medium, and perish at the end of

[1] Cohn's "Beiträge zur Biologie der Pflanzen," Breslau, 1876, Bd. II, S. 300.
[2] *Compt. rend. Acad. d. sc.*, Paris, 1882, t. XCIV, p. 1605.
[3] *Virchow's Archiv*, Berlin, 1884, Bd. XCVII, S. 502.

a longer or shorter period. When the activity of the phagocytes is impeded by unfavourable influences, *e.g.* high temperature, they exhibit a very feeble reaction, incapable of assuring to the frog that immunity which, under normal conditions, it possesses. The conclusions I have just summarised have raised very lively opposition from a large number of observers. Baumgarten[1], with his pupils Petruschky[2] and Fahrenholtz[3], have endeavoured to demonstrate that phagocytosis plays no part in immunity and that the frogs resist anthrax simply because the bacilli are incapable of maintaining themselves alive in the fluids of this Batrachian. Nuttall[4], of Flügge's school, also maintained that frogs resist anthrax owing to the bactericidal power of their fluids. This view has been defended by several other observers and appeared for some time to become quite dominant.

Nevertheless, it is possible to demonstrate that the plasmas of the frog not only are not inimical to the life of the bacillus, but serve as a good culture medium for it[5]. All that is necessary for the demonstration of this fact is to introduce below the skin of frogs
[147] anthrax spores enclosed in a sac of reed pith, or simply enveloped in a small piece of filter-paper. The plasma of the lymph sac at once permeates the spores and allows them to germinate and produce quite a generation of bacilli. But, as soon as the leucocytes pass through the paper, they seize upon the young bacilli, digest them in their substance and prevent their pathogenic action. The germination of the spores may take place even where they have been introduced below the frog's skin without being protected in any way whatever. But, under these conditions, only a certain number of the spores germinate, the majority not having time to do so before the arrival of the leucocytes. The small, very short bacilli which proceed from the germinated spores, are, along with the spores that have not germinated, soon ingested by the phagocytes. But, whilst the rods are in the end digested within these cells, the ingested spores remain intact for a very long time: they do not germinate, but they are not destroyed and retain their vitality indefinitely, in spite of

[1] *Centralbl. f. klin. Med.*, Bonn, 1888, S. 516.

[2] "Untersuch. über d. Immunität d. Frosches gegen Milzbrand," Ziegler's *Beitr. z. path. Anat.*, Jena, 1888, Bd. III, S. 357.

[3] "Beiträge z. Kritik der Metschnikoff'schen Phagocytenlehre," Inaug. Diss., Königsberg, 1889.

[4] *Ztschr. f. Hyg.*, Leipzig, 1888, Bd. IV, S. 353.

[5] *Virchow's Archiv*, Berlin, 1888, Bd. CXIV, S. 466.

the influence of the phagocytes. It is sufficient to withdraw from a frog, that has been inoculated with anthrax spores some time before and kept at a moderate temperature (15°—25° C.), a little lymph and sow it in any nutrient medium (of those employed in the culture of bacteria), in order to see the spores germinate and produce a whole generation of absolutely normal filamentous bacilli. All these phenomena have been carefully studied by Trapeznikoff[1] in a work executed in my laboratory. It is obvious from his experiments that the phagocytes of the frog are quite capable of protecting the organism against the anthrax bacillus by ingesting and digesting the bacilli in the vegetative state and by preventing the germination of the ingested spores. This phagocytic action is very important in presence of the fact that the plasmas of the frog allow the spores to germinate and the bacilli to develop and produce abundant cultures.

The immunity of frogs against the anthrax bacillus that we have just described and which is guaranteed by the activity of the phagocytes, is constant under the conditions of temperature above mentioned (15°—25° C.), conditions which are sufficient, however, to ensure the death of susceptible cold-blooded animals, such as the cricket or *Hippocampus*, from anthrax. The edible frog, a species that readily accommodates itself to a temperature of 35° C., [148] resists, even under these conditions, infection by the bacillus, as pointed out by Mesnil in a work already cited when treating of the immunity of fishes. The green frog (*Rana esculenta*) when kept for a long time at this high temperature, so suitable for the development of the anthrax bacillus, reacts by the same phagocytic mechanism. The leucocytes of the lymph and blood, the cells of the splenic pulp and Kupffer's stellate cells of the liver, seize the introduced bacilli and digest them as in any other case of phagocytosis. The brown frog (*Rana temporaria*) adapts itself but slightly and with great difficulty to the high temperature and dies whether it has been inoculated with anthrax or not. Under these conditions the bacteria develop in the body of the dead or dying frogs, but Mesnil insists on the fact that a true anthrax infection is not produced, as has been maintained by Gibier as the outcome of his researches.

Dieudonné[2], however, has found a method of removing the natural immunity of the frog against the anthrax bacillus, by inoculating it with an artificial bacterial race which he had adapted to

[1] *Ann. de l'Inst. Pasteur*, Paris, 1891, t. v, p. 362.

[2] *Arb. a. d. k. Gsndhtsamte*, Berlin, 1894, Bd. ix, S. 497.

develop fairly luxuriantly at the low temperature of 12° C. Under these conditions all the inoculated frogs, even those which had resisted the inoculation with ordinary bacteria (grown at 37·5° C.), died within a period of 48 to 56 hours, containing many bacilli in the blood and organs. Dieudonné has not studied the essential mechanism that accompanies this loss of immunity; but it is very probable that, for one thing, we have here to do with a reinforcement, special for the frog, of the bacillus that has become accustomed to develop at a low temperature. This bacillus must multiply, in frogs that have been maintained at a low temperature, much more rapidly and profusely than would the ordinary bacillus. On the other hand, the susceptibility of Dieudonné's frogs must depend on a less resistance of the organism under the conditions of his experiments. Unfortunately, we cannot find in his memoir sufficient data on these points; he does not even state the temperature at which the frogs that had been inoculated with bacteria adapted to cold lived. Dieudonné invokes the analogy of his results with those obtained
[149] in the case of the immunity and susceptibility of frogs as regards a septicaemic bacillus.

This bacillus (*Bacillus ranicida*) has been made the subject of an interesting study by Ernst[1]. It is a small, very slender bacillus, which, in frogs, produces a fatal malady epidemic in spring, but ceasing completely during summer. Taking this fact as a basis, Ernst has succeeded in conferring immunity upon frogs in autumn by placing them in an incubator at 25° C. In spite of the injection of a considerable dose of the small bacillus, the frogs living at this temperature remained in good health, whilst control animals exposed to a low temperature died of septicaemia. The counter-test was made in summer. Inoculated frogs that were kept in the laboratory were unaffected, whilst those that had been kept in a refrigerating apparatus at 6°—10° C. invariably died. It may be asked, Is this evident influence of temperature on immunity and receptivity exercised on the organism of the frog or upon the pathogenic bacillus? In the case where a bacillus can only develop at low temperatures its harmlessness at the higher temperature may be readily understood. The experiments of Ernst have demonstrated, however, that this small bacillus develops much better at 22° C., and even at 30° C., than at lower temperatures. It must be concluded, therefore, that the high temperature which confers immunity acts not by

[1] *Ziegler's Beitr. z. path. Anat.*, Jena, 1890, Bd. VIII, S. 203.

weakening the bacillus, but rather by reinforcing the resisting power of the organism. The low temperatures (6°—10° C.) that are favourable to a fatal infection have a different action; that is to say, they weaken the reaction of the inoculated frogs.

Although Ernst has not studied the mechanism of this resistance fully, it is evident, from the data he has supplied, that it consists in a phagocytic reaction. He was able to demonstrate the ingestion of the bacilli by the phagocytes in the susceptible refrigerated frogs, as well as in the refractory frogs, kept at a higher temperature; but in the former case the phagocytosis was so feeble that 24 hours after inoculation a considerable number of free bacilli were still found in the lymph of the dorsal sac, whilst in the refractory frogs the much more active phagocytosis brought about the disappearance of the free bacilli during the first day. If, as is very probable, the analogy of this septicaemia with anthrax in frogs, upon which Ernst insists, really exists, it must be concluded that the susceptibility of [150] these Batrachians to the modified race of the bacillus depends on their weak phagocytic resistance.

Since, in these two examples of natural immunity in the frog, we have seen that the phagocytic activity exhibits itself in an active form against bacteria which readily develop in the fluids of the same animal, we might conclude that the reaction of the phagocytes constitutes a general mode of defence in cold-blooded animals. But Lubarsch[1], a very cautious observer, has expressed an opposite view, based on his studies on the bacillus of mouse septicaemia. He convinced himself that frogs will resist injections of even considerable quantities of this bacillus, without any co-operation on the part of the phagocytes. As we have, here, to do with a matter of fact, Mesnil (*l.c.*) set himself to verify these observations, with the object of establishing whether it was a case of a real exception or of a simple misunderstanding. He was able to demonstrate, by irrefutable observations and experiments, that the bacilli of mouse septicaemia when inoculated into frogs, set up a very pronounced positive chemiotaxis on the part of the phagocytes, which seized and digested the bacilli just as they do the anthrax bacillus. This apparent exception, therefore, becomes transformed

[1] *Centralbl. f. Bakteriol. u. Parasitenk.*, Jena, 1889, Bd. VI, SS. 481 and 529; *Fortschr. d. Med.*, Berlin, 1890, Bd. VIII, S. 665; *Ztschr. f. klin. Med.*, Berlin, 1891; "Ueber Immunität u. Schutzimpfung," Schneidemühl's *Thiermed. Vorträge*, 1892, Bd. II.

into an additional argument in favour of phagocytic reaction being a general factor in immunity. In support of this hypothesis I may adduce a further example, already mentioned in a preceding chapter when discussing another question. The frog is very refractory against the cholera vibrio. When these vibrios are inoculated into the dorsal lymphatic sac or into any other part of the body the animal retains its health unimpaired. An examination of the exudation at the point of inoculation demonstrates that the vibrios meet with a vigorous opposition on the part of the phagocytes, which ingest and completely digest them. This is of special interest from the fact that the frog is very sensitive to the toxin of the cholera vibrio. When injected in a weak dose it kills the frog very quickly. Two small frogs died in less than an hour from the effect of 0·5 c.c. of cholera toxin.

The natural immunity of the frog against the cholera vibrio affords,
[151] then, an example in which the organism, destroying the vibrio by phagocytosis, prevents the production of the poison, which, otherwise, would infallibly kill it.

Having demonstrated that phagocytic reaction manifests itself in the frog in all cases of natural immunity that have been sufficiently studied, we must dwell for an instant on the question of the condition of the bacteria at the moment of their ingestion by the phagocytes. It is very evident that this phagocytic defence is only efficient on condition that it is exercised against bacteria which, in its absence, might injure the organism by their multiplication and their virulence. For this reason the question as to whether the micro-organisms, before being ingested, were living and capable of producing their pathogenic action has been widely discussed. It has even been suggested that the phagocytes are only capable of ingesting the dead bodies of micro-organisms that have been killed by other agents. Frogs are very suitable for a study of this question. When a drop of the exudation is removed some time after inoculation with a motile organism, such as the *Bacillus pyocyaneus* or the cholera vibrio, the organism was often found moving rapidly within the vacuoles inside leucocytes. The experiment will succeed even more completely if a drop of frog's lymph be mixed, on a slide, with a trace of a culture of these motile micro-organisms, the latter being soon found in the clear vacuoles included in leucocytes and executing extremely rapid movements.

Besides this direct proof we can assure ourselves of the living

condition of the micro-organisms in another way. Withdraw a drop of the exudation at an advanced stage of the process when there are no longer any free micro-organisms; inside the phagocytes a few scattered bacteria, more or less well preserved, can still be seen. It is sufficient to keep a hanging drop of such an exudation at a temperature of about 30° C., care being taken to keep it from drying, but without adding to it any nutrient medium. Under these conditions the leucocytes die more or less rapidly, but the bacteria regain vigour: they begin to multiply, and at the end of a short time produce a generation of bacteria within the dead leucocyte. The multiplication of the bacteria goes on progressively and the hanging drop is transformed into a real pure culture. Mesnil was able to confirm these data with the exudations of frogs that had been inoculated with either the bacilli of anthrax or of mouse septicaemia.

The bacteria, ingested in the living state by phagocytes, retain [152] their original virulence. Some authors think, and I was formerly of this opinion, that at the end of a more or less prolonged sojourn within the leucocytes, anthrax bacilli undergo an attenuation in their virulence. Later, numerous researches have, however, demonstrated that this opinion is incorrect, and that the virulence is maintained in the bacteria included in the phagocytes of frogs the whole time that these bacteria remain alive. Dieudonné has insisted on this fact as regards the anthrax bacillus. Mesnil has confirmed it for this same species and for the bacillus of mouse septicaemia. It is impossible, therefore, to doubt this general result, that frogs which are refractory against certain bacteria resist because of the phagocytosis which is exercised against living and virulent micro-organisms.

We have insisted sufficiently on the analysis of the natural immunity of the frog, and need not tarry over the facts relating to other amphibia which, moreover, have been much less studied. The reptiles, those higher representatives of the Vertebrata called cold-blooded, often present examples of really remarkable immunity. Thus alligators will resist enormous doses of various bacteria, such as the anthrax bacillus, that of human tuberculosis or the cocco-bacillus of typhoid fever. When, some time after an injection is made, the exudation at the point of inoculation is withdrawn there is found a large number of leucocytes, amongst which may be recognised many eosinophile microphages, though the majority are macrophages with one, two or more nuclei. Really giant cells are found in the

exudation. It is the macrophages which specially manifest phagocytosis and they are often found crammed with the injected bacteria, as I was able to assure myself after injections of typhoid cocco-bacilli. The natural immunity of alligators (*Alligator mississipiensis*) persists not only at the temperature of the incubator (37° C.), but also at room temperature (20°—22° C.).

Passing in review the animal kingdom we must pause for a moment to consider the natural immunity of birds or lower warm-blooded Vertebrates. The classic example of this immunity is that of the fowl against anthrax. It has long been known that birds resist
[153] inoculation with anthrax or only exhibit a feeble receptivity; though smaller birds are for the most part susceptible to anthrax, the pigeon is much less so and the fowl presents a case of the most pronounced immunity. It was believed to be absolutely refractory until the experiments of Pasteur and Joubert[1], who found a sure method of suppressing this immunity. Fowls that had been inoculated with the bacillus were immersed up to the thighs in cold water in order to bring down their temperature. It was found that, under these conditions, the anthrax bacillus developed at the seat of inoculation and later became generalised in the blood, and invariably caused death. It was concluded from this that the natural immunity of the fowl was dependent on its very high normal temperature (41°—42°) which interfered with the pathogenic functions of the anthrax bacillus.

Hess[2] studied the mechanism of this immunity of the fowl and pointed out the important part that phagocytosis plays in the destruction of the inoculated bacteria.

These researches were resumed in my laboratory by Wagner[3]. Having established that the anthrax bacillus develops readily in the blood and the blood serum of fowls, outside the organism, at high temperatures (42°—43° C.), he came to the conclusion that the lowering of the temperature of the body of the fowls by immersing them in water produced, not a reinforcement of the bacillus, but a weakening of the resisting power of the animal. He was able to convince himself that this resistance exhibits itself in the activity of the phagocytes which ingest and destroy the anthrax bacillus in its vegetative state. In the normal fowl the phagocytosis is rapid and very pronounced, whilst in a fowl that has been refrigerated this

[1] *Bull. Acad. de méd.*, Paris, 1878, p. 440.
[2] *Virchow's Archiv*, Berlin, 1887, Bd. CIX, S. 365.
[3] *Ann. de l'Inst. Pasteur*, Paris, 1890, t. IV, p. 570.

reaction is very slight or absent. To corroborate this general conclusion, Wagner, instead of lowering the temperature by means of cold water, made use of antipyrin and chloral. The application of this treatment likewise caused enfeeblement of the natural defence of the organism and suppressed the immunity of the fowl against anthrax.

Trapeznikoff[1] has studied carefully the fate of anthrax spores when injected into fowls. He observed that most of them are devoured by the leucocytes. Some of the spores were first trans- [154]
formed into small rods, sometimes growing into real bacilli, but finally they all became the prey of phagocytes and perished in their interior. Those in the vegetative condition are soon digested, the spores, however, persist for some time inside the phagocytes, but ultimately disappear. The phagocytosis in fowls inoculated with spores is very marked, and preparations, stained by Ziehl's method, demonstrate most clearly the reality of this reaction phenomenon. These preparations have for long been used in the course in bacteriology at the Pasteur Institute for the demonstration of phagocytosis.

In the face of these facts, well established and confirmed many times, it is impossible to accept Thiltges'[2] denial of the ingestion of these bacteria by the phagocytes of the fowl. Some fault of technique, which I am not at the moment in a position to indicate exactly, has evidently slipped into this author's work. The positive data, however, on phagocytosis in the fowl, obtained by Hess, Wagner, and Trapeznikoff, data confirmed by myself, render unnecessary any fresh researches for the purpose of explaining the negative results obtained by Thiltges. As regards his experiments on the bactericidal action of defibrinated blood and of blood serum of fowls against the bacillus and its spores, experiments whose results are opposed by those of Wagner, the contradiction may be explained pretty easily, at least in part. Thiltges mentions several times that the bacilli, when sown in the blood serum of the fowl, were aggregated in clumps. Nevertheless, he has failed to guard against this source of error and has attributed the diminution in number of the colonies on plates to the destruction and not to the agglutination of the bacilli. Thiltges gives so few particulars of the conditions under which his experiments were performed that we do not even know at what temperature

[1] *Ann. de l'Inst. Pasteur*, Paris, 1891, t. v, p. 362.
[2] *Ztschr. f. Hyg.*, Leipzig, 1898, Bd. xxviii, S. 189.

he kept his tubes containing blood and serum sown with bacilli. As Wagner kept his at 42°—43° C., a temperature which corresponds to that of the body of the fowl, I asked M. Gengou to make a series of experiments on the bactericidal power of the plasma and blood serum of fowls on the anthrax bacillus, keeping his tubes at 37° C.
[155] The result of his experiments was in complete accord with those of Wagner. Under the conditions that I have just stated the fluids of the fowl are no more bactericidal than they are under the conditions maintained in Wagner's experiments.

In summing up these data on the natural immunity of fowls against anthrax, we are certainly justified in concluding that it is due to the phagocytosis and not to any bactericidal property of the "humours."

The pigeon is more susceptible than the fowl to the action of the anthrax bacillus, still it manifests a certain degree of resistance against the microbe. After what we have said on the subject of the fowl we need make but few remarks on the pigeon, in spite of the very animated discussions that have taken place on the mechanism of its immunity. When Baumgarten was offering a systematic opposition to the part played by phagocytic reaction in immunity, he set his pupil Czaplewski[1] to investigate the resistance of pigeons against anthrax. The results of this investigation were absolutely negative as regards phagocytosis. The latter was said to have no importance in the defence of the organism, which resisted simply because it was impossible for the bacillus to live in the body of the pigeon. I then set myself to study this question[2], and I was able to demonstrate that the anthrax bacillus is quite capable of keeping alive in the pigeon, that it can develop in its fluids, but that it is unable to defend itself against the aggression of the phagocytes which ingest it and completely digest it. By isolating the phagocytes that had ingested the bacilli injected into the body of the pigeon, I was able to prove that a number of these bacilli were still alive. The enfeeblement and death of the phagocytes when outside the body allowed the anthrax bacilli again to get the upper hand in this struggle, to develop and to give virulent cultures. The part played by phagocytes in this example of natural immunity was thus placed beyond doubt.

[1] "Untersuchungen ü. die Immunität d. Tauben," Königsberg, 1889; *Ziegler's Beitr. z. path. Anat.*, Jena, 1890, Bd. VII, S. 49.

[2] *Ann. de l'Inst. Pasteur*, Paris, 1890, t. IV, p. 38; p. 65.

Later, Czaplewski[1] himself became convinced that his previous negative results would not stand criticism, and Thiltges, in his work already mentioned, when discussing the fowl, was able to confirm the [156] importance of phagocytosis in the defence of the organism of the pigeon against anthrax. He was struck by the difference between these two species of birds. In the pigeon it was easy for him to prove that in the individuals that succumb to anthrax the phagocytic reaction is very feeble, whilst in those which ultimately resist the bacillus it is very pronounced. Thiltges likewise observed that the blood and blood serum of pigeons when sown *in vitro* with the anthrax bacillus, manifest only an insignificant bactericidal power, a fact that further warrants him in attributing great importance to phagocytosis in the maintenance of the natural immunity of the pigeon. It is remarkable that, in presence of these facts, it did not occur to the author to ask whether this fundamental difference in the mechanism of the resistance, which he thought possible in two birds so closely allied as are the pigeon and the fowl, really did exist in nature. I infer that his experiments on the fowl were made before those on the pigeon, and that the difference in his results depended specially on the fact that he had acquired greater skill in executing his later experiments.

Having observed that frogs die readily when inoculated with an anthrax bacillus that was adapted to develop at a low temperature, Dieudonné (*l.c.*) endeavoured to suppress the immunity of the pigeon by using bacilli adapted to a high temperature. But the inoculation of a second generation of the anthrax bacillus, cultivated at 42° C., was borne by five pigeons without inconvenience. Even bacilli that were rendered capable, by cultivation through sixteen generations, of developing at this temperature were not in a condition to kill more than five pigeons out of thirteen inoculated. These attempts to explain immunity as due to the properties of the bacilli rather than to those of the organism of the pigeon, have therefore led to a result very different from that anticipated by Dieudonné.

The pigeon is further of interest to us because of its natural immunity against the bacillus of human tuberculosis. It resists considerable doses of this bacillus, so virulent for man and for the majority of mammals, and even for some birds (canaries and parrots). Dembinski[2], studying the mechanism of this immunity, was able to

[1] *Ztschr. f. Hyg.*, Leipzig, 1892, Bd. XII, S. 348.

[2] *Ann. de l'Inst. Pasteur*, Paris, 1899, t. XIII, p. 426.

prove that the bacilli of human tuberculosis encounter in the organism of the pigeon a very great resistance from the phagocytes,
[157] especially from the macrophages. These cells fuse together around masses of bacilli and imprison them within real giant cells or polynucleated macrophages (Fig. 21). The microphages in this struggle play only a secondary part, but the resistance offered by the macrophages is a most effective one. Incapable of completely destroying the bacilli, these phagocytes exercise over them an unfavourable influence and prevent them from multiplying and exhibiting their noxious action. The importance of the defence by the macrophages comes out still more clearly when compared with what takes place if, instead of the bacillus of human tuberculosis, we inoculate into pigeons the bacillus of avian tuberculosis. In the latter case the microphages certainly promptly seize the bacilli, but being powerless against them they perish, whilst the macrophages only intervene later on and in small numbers. The result is that in the pigeon the avian bacillus becomes generalised in the organism and sets up a fatal tuberculosis.

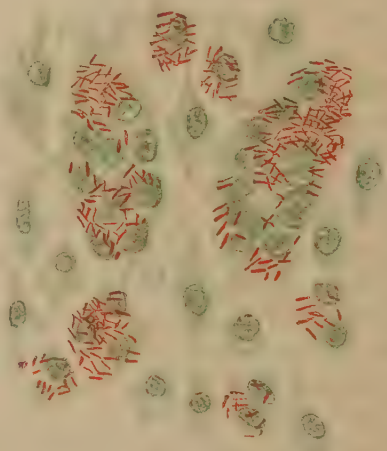

Fig. 21. Reaction of the phagocytes of the pigeon against the bacilli of human tuberculosis.

It must be admitted, then, that the immunity of the pigeon against the bacillus of human tuberculosis is due to the defence by the macrophages. This conclusion is corroborated by the fact that in the fowl—equally refractory against the same bacillus—there is also observed a very strong macrophagic reaction.

Nocard[1], who for several years has been carrying on studies on the relations between the bacilli of human and avian tuberculosis, conceived the idea of adapting the former to the organism of the

[1] *Ann. de l'Inst. Pasteur*, Paris, 1898, t. xii, p. 561.

fowl. With this object he enclosed a culture of the bacillus of [158]
human tuberculosis in a sac of collodion which he then introduced into the peritoneal cavity of fowls. Under these conditions the bacillus, protected against the aggression of phagocytes, continued to live inside the sac through whose walls the fluid part of the peritoneal lymph could diffuse. After several passages from sac to sac the human bacillus becomes acclimatised to the body of the fowl and is transformed into a variety quite comparable to the bacillus of avian tuberculosis. This experiment has definitely settled the question so long under discussion of the specific difference between the two tubercle bacilli. It has resolved it in the sense of affirming their unity; the avian bacillus is only a modified race of the same bacillus which sets up tuberculosis in man and other mammals.

In spite of the great difference between the anthrax bacillus and that of human tuberculosis, the immunity against these two bacteria, which is shown in birds, depends in every case upon the reaction of the phagocytic system.

Having rapidly glanced at natural immunity as we ascend the scale of the animal series we now come to it as it presents itself in the highest class, Mammals, a question on which it is necessary to dwell at greater length because of its great importance, and also because of the fuller study that has been given to it.

As the immunity of the Invertebrata and of the lower Vertebrata against the anthrax bacillus has furnished us with several important indications we will first endeavour to throw light on the mechanism of the resistance offered to anthrax by certain mammals. The representatives of this class being, however, for the most part extremely susceptible to this disease, examples of true natural immunity are very rare. The first place among resistant mammals is occupied by the dog. Although young dogs, as demonstrated by Strauss[1], readily take fatal anthrax, the canine species may nevertheless be regarded as possessing a real immunity, as adult dogs withstand, without inconvenience, the inoculation of large quantities of bacilli. When introduced beneath the skin these bacilli excite a local inflammation, accompanied by a very marked diapedesis of white corpuscles which at once begin to devour the bacilli. This phagocytosis has already been observed by Hess[2], Malm[3], myself, and several other investigators, [159]

[1] *Arch. de méd. expér. et d'anat. path.*, Paris, 1889, t. I, p. 325.
[2] *Virchow's Archiv*, Berlin, 1887, Bd. CIX, S. 365.
[3] *Ann. de l'Inst. Pasteur*, Paris, 1890, t. IV, p. 520.

so that its existence cannot be doubted. Recently, Martel[1] has demonstrated a very distinct phagocytic reaction in all those cases where he has had to deal with dogs that were refractory or not very susceptible. This reaction is shown by the ingestion of the bacteria and by the large accumulation of leucocytes at the seat of inoculation. His researches are of special interest by reason of the counter-test that he was able to make upon dogs that were susceptible to anthrax. It was demonstrated some years ago that the natural immunity of the dog against the bacillus, although very real, is, nevertheless, relative and limited. Thus Bardach[2] established the fact that dogs from whom the spleen, an organ full of phagocytes, had been removed, became susceptible to anthrax. Even dogs into whose veins he injected fine wood-charcoal powder suspended in water, with the purpose of "diverting" the phagocytosis, readily succumbed to anthrax.

Martel endeavoured to suspend the natural immunity of dogs by injecting into them phloridzin or pyrogallic acid. But he obtained much more constant results by inoculating the bacillus into rabid dogs. The organism, weakened by this terrible disease, became very susceptible to anthrax, and the rabid animal succumbed to anthrax before the rabies had completed its evolution. By its passage through the rabid dog the anthrax virus is so augmented in virulence that it becomes fatal for normal dogs. Martel succeeded also in reinforcing the bacillus isolated from a cow affected with anthrax. In all these cases where the reinforced bacilli set up a severe and rapidly fatal infection, Martel could demonstrate only a feeble phagocytic reaction.

Researches on the phagocytosis of dogs, inoculated with the anthrax bacillus, have always demonstrated a regular and constant relation between this reaction and the resistance of the organism. On the other hand, experiments undertaken for the purpose of establishing the part played by the body fluids in this immunity, have always given negative results.

As the dog, of all mammals, exhibits the greatest natural immunity from anthrax, it is very natural that in the bactericidal property of its blood the key to the enigma has been sought. Thus
[160] Nuttall[3] concludes from his experiments that the anthrax bacillus

[1] *Ann. de l'Inst. Pasteur*, Paris, 1900, t. XIV, p. 13.
[2] *Ann. de l'Inst. Pasteur*, Paris, 1889, t. III, p. 577.
[3] *Ztschr. f. Hyg.*, Leipzig, 1888, Bd. IV, S. 353.

is readily destroyed by defibrinated dog's blood. But, as this result was not in accord with my observations[1] that the bacillus is easily cultivated in dog's blood, and as several observers, especially Lubarsch[2], had arrived at conclusions opposed to those of Nuttall, systematic researches were made for the purpose of solving this complicated problem. Denys and Kaisin[3] sought to remove the objections formulated against the explanation of the immunity of the dog as due to the bactericidal property of its blood by affirming that this power, which is absent in the inoculated dog, develops whilst the animal is under the influence of the bacillus. Immunity is reduced, then, in this case to the establishment of a new property in the fluids during the course of the struggle of the organism against the inoculated bacillus. None of the observers, however, who have repeated these experiments, *e.g.* Lubarsch[4] and Bail[5], were able to confirm the results of the Belgian observers. Denys himself, indeed, having resumed this study with Havet[6], had to reject the conclusions of his former work executed in collaboration with Kaisin. He is persuaded that their error was due to the fact that in their experiments *in vitro*, the living leucocytes ingested the bacilli and prevented their development. As the result of these new researches Denys and Havet have come to the conclusion "that the main, the predominating part of the bactericidal power of the dog's blood must be ascribed to the leucocytes acting as phagocytic elements" (*loc. cit.* p. 15).

As a result of the investigations I have summarised the conclusion is forced upon us that the natural immunity of the dog from anthrax is a function of the phagocytes. In presence of this uniformity of the experimental results it becomes very important to make a more profound study of the phenomena that manifest themselves during the destruction of the bacilli by the phagocytes of the dog. What are the phagocytic elements which play the principal part in this struggle, and by what means do they attain this result? Gengou[7] undertook a [161]

[1] *Ann. de l'Inst. Pasteur*, Paris, 1887, t. I, p. 43.

[2] "Untersuchungen ü. die Ursachen der angeborenen u. erworbenen Immunität," Berlin, 1891, S. 111.

[3] *La Cellule*, Lierre et Louvain, 1893, t. IX, p. 337.

[4] "Zur Lehre von den Geschswülsten und Infectionskrankheiten," Wiesbaden, 1899.

[5] *Centralbl. f. Bacteriol. u. Parasitenk.*, 1^te^ Abt., Jena, 1900, Bd. XXVII, SS. 10 und 517.

[6] *La Cellule*, Lierre et Louvain, 1894, t. X, p. 7.

[7] *Ann. de l'Inst. Pasteur*, Paris, 1901, t. XV, p. 68.

detailed investigation in my laboratory to answer these questions. He was able to convince himself, in agreement with the statements of his predecessors, that not only was the serum of dog's blood not bactericidal for the anthrax bacillus, but that the plasma of the blood is no more so. The fluid of the aseptic pleural exudation obtained after injection of gluten-casein, was likewise incapable of killing the anthrax bacillus. When Gengou, by means of centrifugalisation, isolated the leucocytes from these exudations, washed them in physiological salt solution, froze them, and then macerated them in broth, he obtained suspensions of white corpuscles, to which he added bacilli. He was able to demonstrate that when the exudations contained macrophages principally, as is observed in exudations taken at the end of two or three days, the bactericidal power of the suspensions was *nil* or insignificant. When, on the other hand, the leucocytes came from exudations only twenty-four hours old and were composed almost exclusively of microphages, the destructive action on the bacilli of the extract of the microphages in broth was most marked. Now it is fully demonstrated that in the exudation set up in the refractory dog by the injection of anthrax bacilli, it is especially the microphages which exhibit the phagocytic reaction against this bacillus.

This is how the question of the immunity of the dog from anthrax stands at present. The natural immunity of this species, which although not unlimited, is very real, depends on the activity of phagocytes. These elements, under the stimulus of the bacillus and its products, exhibit a positive chemiotaxis of the most marked character, they approach the bacilli, ingest them by a physiological act, and destroy them by means of a substance which is not found in either the plasma or the blood serum, but which can be demonstrated in an extract of the microphages.

In spite of the uniformity and precision of these data, it is impossible to rest satisfied with describing, as an example of natural immunity from anthrax, the single case of the dog. If the resistance of the rat against this disease was merely of historical interest because of the large number of works devoted to this question, we might
[162] relegate it to the chapter reserved for the history of our knowledge on immunity. But it is not so. The anthrax of rats is a subject full of very valuable instruction, and von Behring was quite justified in saying that whoever wished to get a true conception of natural immunity from a virus should pay special attention to this example.

As a matter of fact, it may be stated that the grey rat (*Mus decumanus*), the black rat (*Mus rattus*), and white rats are far from enjoying a true immunity from anthrax. They, nevertheless, exhibit a more or less marked resistance against this disease and are always less susceptible than are the other laboratory rodents: mice, guinea-pigs and rabbits. Rats resist attenuated bacilli (anthrax vaccines) better than do these three species, and in order to induce in them fatal anthrax it is necessary to inoculate a much larger number of virulent bacilli. On the other hand, rats are distinguished by a great irregularity in the resistance they offer to the bacillus. At times they resist very virulent bacilli; at others they contract a fatal disease after an injection of very attenuated bacilli (Pasteur's first vaccine).

In my first memoir on anthrax[1] I noted the fact that in rats the phagocytosis against the bacillus when injected subcutaneously was more marked than after the same inoculation into the rabbit and guinea-pig. Later, this fact was disputed by several observers, who refused to accept the extent and importance of the phagocytic reaction in the rat. This opposition was strengthened by a very interesting discovery made by von Behring[2], namely, that the blood serum of the rat possessed a remarkably destructive power for the anthrax bacillus. When this observer added a certain quantity of anthrax bacilli to some blood serum of the rat, instead of elongating into filaments and dividing they underwent a change, lost their normal refraction and took on staining reagents very imperfectly. The membranes alone remained of the bacilli thus treated. Von Behring considered that this bactericidal action of the serum depends on the presence of an organic base dissolved in the blood fluid. He had merely to neutralise the serum by means of an acid, and there was at once a very abundant development of the bacillus. From these researches von Behring came to the conclusion that the natural immunity of the rat from anthrax can be reduced to terms [163] of the chemical action of the blood on the bacillus.

In one of his most recent publications this author[3] returns to the question of anthrax in rats and sums up his present point of view as follows. He regards the immunity of these rodents as being

[1] *Virchow's Archiv*, Berlin, 1884, Bd. XCVII, S. 516.

[2] *Centralbl. f. klin. Med.*, Bonn, 1888, No. 38.

[3] "Infectionsschutz und Immunität" in Eulenberg's "Real-Encyclopädie d. ges. Heilkunde," III^te^ Aufl. (*Encyclop. Jahrbücher*), Wien, 1900, Bd. IX, S. 196.

relative, not absolute. "The anthrax bacilli"—he says—"die in rat's serum *in vitro*; and in the cases where the inoculation of these animals with the anthrax virus is not fatal, it is at least reasonable to assume that the blood fluid likewise produces this protection in the organism of the living rat. Now, an immunity that manifests itself without the aid of any activity of the cell must undoubtedly be regarded as being of a humoral character" (*loc. cit.* p. 202).

Let us begin by analysing the facts as presented in rats into
[164] whose subcutaneous tissue we have injected anthrax virus. A certain number of them resist, without exhibiting any lesion other than a certain exudative inflammation at the seat of inoculation. The exudation is, in this case, very rich in leucocytes which quickly exert their phagocytic function and destroy the ingested bacilli. In this reaction it is the microphages that play the chief part, the macrophages intervening later and in a much less pronounced fashion. Usually, however, the inoculated rats exhibit a more serious illness: the bacilli multiply at the point of inoculation and excite the formation of an extensive oedema, rich in serous fluid, transparent, and very poor in leucocytes. It is only later that these cells intervene in any considerable number. The exudation becomes thicker and turbid, the numerous white corpuscles devour the bacilli and cause their disappearance. Under the influence of this marked reaction the animals in most cases recover, as has already been established by Frank[1]. But even in those individuals which succumb to anthrax death occurs more or less tardily, an examination of the internal organs then revealing a considerable phagocytic reaction. The spleen, often of enormous size, contains numerous macrophages which are filled with normal or more or less altered bacilli. In the liver these macrophages, which have devoured several microphages and some bacteria, are also found (Figs. 22 and 23).

When instead of bacteria in the condition of rods, anthrax spores are inoculated subcutaneously or into the anterior chamber of the eye, we can observe their germination. There is developed a whole generation of bacilli which behave like those we have already described, that is to say, they excite an exudation and are ultimately digested within the phagocytes (Figs. 24 and 25). All these phenomena of phagocytosis I described in detail more than ten years ago in my memoir on the anthrax of rats[2]. Since then not a single

[1] *Centralbl. f. Bacteriol. u. Parasitenk.*, Jena, 1888, Bd. IV, SS. 710, 737.
[2] *Ann. de l'Inst. Pasteur*, Paris, 1890, t. IV, p. 193.

fact has been brought forward to invalidate the results there set forth.

Fig. 22. Macrophage from the liver of a rat affected with anthrax.

Fig. 23. Macrophage containing bacilli, from the liver of a rat affected with anthrax.

How is this paradoxical fact to be explained, that anthrax which grows in the body of the rat, there setting up a disease more or less grave and sometimes fatal, is so readily destroyed by the serum and

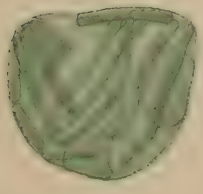

Fig. 24. Microphage of rat filled with bacilli.

Fig. 25. Two microphages of rat that have ingested bacilli.

blood when removed from the organism? From numerous experi-
[165] ments, carried out by Hankin[1] and by Roux and myself[2], it has been demonstrated that the bactericidal power of the fluids of the rat cannot be invoked as the cause of the animal's resistance to anthrax. Those rats which show themselves very susceptible to this disease and die from anthrax infection, furnish, nevertheless, a serum that will prevent anthrax in other rats, and which will protect even mice into which the bacilli have been injected. Rats into which we inoculate on one side of the body a little anthrax culture, and on the other side the same quantity of bacilli mixed with blood serum from the same animal, manifest oedema at the former place only. It is from this latter point that the general infection takes place, the side where the anthrax bacilli mixed with serum was introduced remaining unaffected. Sawtchenko[3], who has investigated the immunity of the rat in my laboratory, has to the facts just mentioned added the observation that when the injection of bacilli causes haemorrhage the rat survives. When, on the contrary, the injection is made with a fine needle and without effusion of blood, the rat contracts a fatal anthrax.

It follows from these facts that the blood, immediately it has
[166] escaped from the vessels, undergoes a change in its composition and becomes bactericidal for the anthrax bacillus, whilst, when it is circulating in the organism, it exhibits no such power. Sawtchenko has studied the substance in the serum which kills the bacilli and has demonstrated that it will resist heating to 56° C.; even when heated to 61° C. the serum still exercises a certain amount of bactericidal power for very attenuated bacilli (Pasteur's first vaccine). Researches on the distribution of this bactericidal power in the living rat have convinced Sawtchenko that none of it passes into the fluid of the passive oedema set up by the slowing of the circulation, nor into that of the active oedema developed as the result of the inoculation of anthrax bacilli. He observed that even the bacillus of Pasteur's first vaccine grows abundantly in the oedematous fluid produced by the injection of virulent bacilli. The peritoneal lymph, however, exerts a very marked bactericidal action on the bacilli. Having demonstrated this fact Sawtchenko put to himself the question: May not the great difference between the action of these fluids depend on the

[1] *Centralbl. f. Bacteriol. u. Parasitenk.*, Jena, 1891, Bd. IX, SS. 336, 372.
[2] *Ann. de l'Inst. Pasteur*, Paris, 1891, t. V, p. 479.
[3] *Ann. de l'Inst. Pasteur*, Paris, 1897, t. XI, p. 865.

fact that the lymph is rich in leucocytes, whilst in the fluid of the oedema they are almost absent? Pursuing this question, Sawtchenko made a comparative study of the bactericidal power of the serum, prepared outside the body, and of the blood plasma obtained by means of an extract of the heads of leeches, and he concluded from his researches that the bactericidal substance circulates in the plasma of the living rat and that it is not derived from the microphages, but must be looked upon rather as a secretion of the macrophages in the blood and of endothelial cells. This result was not confirmed by Gengou[1], who also took up the study of this important question in my laboratory. Instead of preparing the plasma by means of the addition of an extract of leeches he made use of a method much more perfect and free from sources of error. He introduced no foreign substance capable of affecting the results of his experiments. Collecting the rat's blood in paraffined tubes, and centrifugalising it in similar tubes, he obtained a fluid which approaches much more closely the plasma of circulating blood than does serum. This fluid, however, will coagulate at the end of a fairly long interval, which proves that it cannot be looked upon as blood plasma. Gengou examined the bactericidal power of the fluid portion of the "plasma," obtained by the process just described, on the anthrax [167] bacillus, and also that of serum prepared in tubes in the ordinary way. The difference between the two fluids is very marked; whilst the serum destroys the bacilli sown in it very rapidly and dissolves their contents, the fluid of the "plasma" has no similar action. These results, confirmed several times, demonstrate very definitely that the plasma of the circulating blood does not contain any bactericidal substance. This, during the life of the animal, is found inside leucocytes and only escapes from them when the cells burst or undergo profound lesions, this taking place when the clot is formed and when the serum is prepared outside the organism, or in the effused and coagulated blood, or again in the peritoneal lymph during phagolysis. This phagolysis is inevitably produced as a result of rapid injection of foreign fluids into the peritoneal cavity, *e.g.* of broth or of physiological salt solution, containing bacteria in suspension.

The facts we have brought together on the subject of anthrax in rats form a whole whose several parts are in complete harmony. The phagocytes of this species of rodent contain a bactericidal ferment,

[1] *Ann. de l'Inst. Pasteur*, Paris, 1901, t. xv, p. 232.

a kind of cytase, which resists temperatures approaching 60° C. This cytase is very active against the bacilli, but in the living animal it can only act within the phagocytes, or, in a transitory and incomplete fashion, outside these cells, when phagolysis is taking place in the peritoneal cavity. The resistance offered by the rat to anthrax depends, then, on this phagocytic activity. For its manifestation it is necessary, first of all, that the phagocytes should manifest a positive chemiotaxis for the bacilli, and then that they should seize and ingest these organisms. These are the vital acts that decide the result of the struggle. When the phagocytes show themselves inactive the bacilli multiply in the oedematous fluid which contains no bactericidal cytase, and pass into the plasmas of the lymph and of the blood, which also are incapable of killing these bacteria. The animal may, then, die of anthrax, in spite of the presence in its body of a large quantity of bactericidal cytase which is to be found in situations to which the bacilli have not penetrated. In those cases, on the other hand, where the phagocytes accomplish their function, where they rush up to the menaced point and devour the inoculated bacteria, these bacilli are placed in contact with the intracellular cytase and
[168] undergo complete digestion. The organism in this case gets rid of its enemies and victoriously resists infection.

Anthrax in rats, then, presents one of the most instructive examples of natural immunity. But the detailed analysis of the mechanism of this resistance demonstrates very clearly the great part played by the phagocytes in this process. In this respect the organism of the rat presents, in a general fashion, a great analogy to the natural immunity of the dog, of birds, and of other representatives of the animal kingdom that we have examined. Under these conditions it is useless to insist at any length on other examples of resistance against anthrax which, moreover, have relation much more often to a natural immunity against attenuated bacilli than to one against true anthrax virus. Rabbits and guinea-pigs, so sensitive to this virus, often resist the inoculation of Pasteur's vaccines. The rabbit is, in general, refractory to the first anthrax vaccine; it may even resist the second vaccine. The guinea-pig, a more sensitive animal, does not exhibit any natural immunity except against the first vaccine. In all these cases the mechanism is similar to that which the rat and the dog oppose to virulent anthrax. The bacilli, into whatever part of the body they are injected, set up an exudative inflammation which brings up a large number of leucocytes to the

point menaced. These cells readily exert their phagocytic function and rid the organism of the introduced bacteria. In order to obtain a complete grasp of the part played by this reaction it will be found useful to inject beneath the skin of one ear of a rabbit a little anthrax vaccine and beneath the skin of the other the same quantity of virulent bacilli. The difference between the reaction in the two cases is very striking. The ear inoculated with vaccine soon becomes the seat of a circumscribed inflammation with a purulent exudation, all the bacilli in which have been devoured by the leucocytes. The other ear, on the contrary, presents, around the injected virus, only a serous or blood-tinged exudation containing no, or few, leucocytes ; the bacilli are found free in the liquid and multiply without let or hindrance. Meeting with no opposition the virus becomes generalised throughout the organism and brings on death by anthrax septicaemia. Rabbits, into which anthrax vaccines only are introduced, oppose to the invasion of the bacilli a leucocytic barrier which arrests their extension. The natural immunity of the sheep, rabbit and guinea-pig is also a phagocytic immunity, but it is only capable of being exercised against bacilli previously attenuated in virulence. The [169] researches of Mme Metchnikoff[1] on the reaction of the phagocytes of these animals to the bacilli of Pasteur's two anthrax vaccines have demonstrated the importance of the destruction of these bacilli by the leucocytes. All the other examples of natural immunity against anthrax are also merely relative. The fowl that resists an anthrax virus strong enough to kill an ox or a horse, succumbs to a special variety of anthrax cultivated by Levin[2]. The dog, as we have seen, in spite of its pronounced natural immunity against anthrax, is killed by the special anthrax bacillus prepared by Martel.

In this immunity against anthrax we have to deal with a bacillus capable of living and reproducing itself in extremely varied media. Hence the reason, it may be said, that the bactericidal influence of the fluids is so little pronounced in this case. To bring it into relief we must, therefore, choose a bacterium less capable of adapting itself to the chemical composition of various culture media. In this matter we cannot do better than select pathogenic spirilla of extremely delicate nature and analyse the mechanism of the natural immunity of certain species of animals with respect to them. It must not be forgotten, however, that here we are making use of

[1] *Ann. de l'Inst. Pasteur*, Paris, 1891, t. v, p. 145.
[2] "Om Mjältbrand hos Höns," Stockholm, 1897.

representatives of an infinitely small minority of pathogenic bacteria, the majority resembling the anthrax bacillus in the facility with which they can be cultivated in all sorts of nutritive media.

The spirillum of recurrent fever of man (*Spirochaete obermeyeri*) was the first pathogenic microbe found in an infective disease distinctly human. Discovered a third of a century ago, it has passed through the hands of the most skilful bacteriologists, who have tried all possible methods of cultivating it outside the body. Koch himself tried to solve the problem, but, in spite of his incomparable skill, did not succeed. Later, Sakharoff[1], at Tiflis, discovered a spirillum very similar in appearance which produced a fatal septicaemia in the goose. He, also, tried to cultivate it, but in vain. His successors have not been more fortunate in this respect. Here, then,
[170] are two micro-organisms, against which natural immunity should be easily obtainable and in a fashion quite other than that against anthrax. Nothing, indeed, is more frequent than examples of very stable natural immunity against the spirilla of Obermeyer and of Sakharoff. As I wished to obtain a clear idea of the mechanism by which the guinea-pig resists injections of the spirillum of goose
[171] septicaemia (*Spirochaete anserina*) I made injections of goose's blood, containing a quantity of these organisms, into the peritoneal cavity of guinea-pigs. This injection, as usual, causes the disappearance of most of the leucocytes, as the result of a very marked phagolysis. We know that, under these conditions, the damaged leucocytes allow a certain quantity of the bactericidal cytase to escape. In spite of this the spirilla remain intact and exhibit very active movements in the peritoneal exudation. This exudation, after a period of phagolysis, which lasts for two or three hours, begins to be stocked again with leucocytes which come up in increasing numbers, a fact that does not prevent the spirilla moving about with great rapidity. Even seven hours after the injection of goose's blood we still find many extremely active spirilla among a large number of recently migrated leucocytes, some of which even at this stage contain red corpuscles of the blood of the goose. It is not until later that the ingestion of these spirilla by the leucocytes commences, the leucocytes at last damaging and completely destroying them. This act of phagocytosis may be readily observed in hanging drops of the peritoneal exudation of inoculated guinea-pigs. The attention of the

[1] *Ann. de l'Inst. Pasteur*, Paris, 1891, t. v, p. 564.

observer is drawn to certain macrophage leucocytes which throw out one or two conical-looking processes (Figs. 26—28). These pseudopodia

Fig. 26.—Leucocyte of guinea-pig in the act of ingesting two spirilla.

Fig. 27.—The same leucocyte, half an hour later.

Fig. 28.—The same leucocyte, ten minutes later than Fig. 27.

attach themselves to spirilla which exhibit very violent movements as though wishing to extricate themselves from the grasp of the leucocyte. Sometimes the spirillum succeeds in escaping, but usually

Fig. 29.—Leucocyte of guinea-pig in the act of ingesting a very active spirillum.

Fig. 30.—The same leucocyte, forty minutes later.

Fig. 31.—The same leucocyte, half an hour later than Fig. 30.

it becomes surrounded by the protoplasm and sinks more and more deeply into the substance of the leucocyte. Even when almost surrounded the free part of the spirillum still continues to move (Figs. 29—31). These movements cease only after the complete

ingestion of the spirillum. Once inside the phagocyte the spirillum is digested and soon becomes unrecognisable.

Recently, Sawtchenko[1] took advantage of an epidemic of recurrent fever at Kazan to make similar investigations on the natural immunity of the guinea-pig against Obermeyer's spirillum. He observed that these organisms, when injected into the peritoneal cavity, remained there, alive, for 24 and even 30 hours, whilst these same spirilla, when kept at 37° C. outside the organism in their natural medium, died at the end of some (4—7) hours. The injection of
[172] human serum containing spirilla into the peritoneal cavity of guinea-pigs set up a phagolysis succeeded by a considerable afflux of leucocytes. In spite, however, of the arrival of quite an army of these cells, the spirilla continued to move rapidly; for a long time they evaded the phagocytes which, however, in the end always ingested them. But it is only the macrophages which fulfil their phagocytic function (Figs. 32 and 33); the microphages obstinately

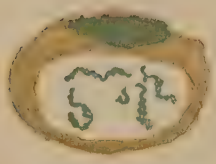

Fig. 32.—Macrophage of guinea-pig filled with spirilla of recurrent fever (after Sawtchenko).

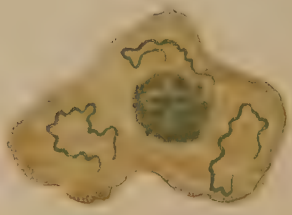

Fig. 33.—Macrophage of guinea-pig containing three *Spirochaete obermeyeri* (after Sawtchenko).

exhibit an absolutely negative chemiotaxis. Now, as the macrophages do not make their way into the peritoneal cavity until after the microphages have appeared, it is easy to understand that phagocytosis can only take place at a late period. Sawtchenko came to the conclusion that "in the peritoneal cavity of animals naturally refractory, the spirochaetes perish as the result of a slow phagocytosis and not from the action of the bactericidal substances of the fluids."

[1] *Arch. russes de pathol.* etc., St Pétersb., 1900, t. IX, p. 578; and Sawtchenko et Melkich, *Ann. de l'Inst. Pasteur*, Paris, 1901, t. XV, p. 502.

In conformity with this result this observer has often noted the ingestion of living spirilla by the macrophages, in hanging drops of the peritoneal exudation of inoculated guinea-pigs. The phenomenon corresponds exactly to that described in connection with the spirillum of the goose.

In spite of the great difference between the spirillum and the anthrax bacillus from the point of view of their adaptation to surrounding media, the general result is the same with both these microbes: animals endowed with natural immunity get rid of them through the agency of their phagocytes.

It would be impossible and even useless here to pass in review [173] all the cases of natural immunity against infective micro-organisms. We must consequently limit ourselves to several examples which may have an interesting bearing on the study of the problem as a whole. The spirilla, whose history we have just recorded, remain in the peritoneal fluid, without change of form, up to the moment when they are captured by the macrophages. Let us see by what mechanism the natural immunity against micro-organisms, characterised by a very special sensitiveness to external influences and by a considerable change of shape, is produced. The cholera vibrio and its allies best satisfy this postulate. When they find themselves placed under unfavourable conditions, these vibrios immediately become transformed into small spherical bodies which are much more like cocci than vibrios. The cholera vibrio is pathogenic for the laboratory rodents, especially for the guinea-pig, when a fairly large quantity of a culture is injected into the peritoneal cavity. Against smaller doses, however, the natural immunity is a most marked one. If we take a race of the cholera vibrio of medium virulence, and inject into the peritoneal cavity of guinea-pigs a sublethal dose of a culture, the following phenomena may be observed[1]. The inoculated vibrios move actively in the peritoneal fluid, from which almost all the leucocytes have disappeared. There remain only a few lymphocytes which appear to be indifferent to the influences that set up a real phagolysis. But, little by little, fresh leucocytes come into the exudation and engage in a struggle with the vibrios which, so long as they are free, retain their curved form and complete motility. The microphages, especially, swarm into the peritoneal cavity. Some of them begin to ingest vibrios, but this phagocytosis is at first slight. Later it becomes much more active. The microphages and macro-

[1] *Ann. de l'Inst. Pasteur*, Paris, 1895, t. IX, p. 448.

phages seize vibrios that are evidently living and uninjured, which, sometimes, may be observed inside the vacuoles of the leucocytic contents exhibiting very lively movements. Once ingested, however, many of the vibrios become transformed into round granules. This change of shape is constant when inside microphages, but is completely absent when inside macrophages (Figs. 34 and 35). Finally,

Fig. 34.—Microphage of guinea-pig filled with cholera vibrios, the majority of which are transformed into granules.

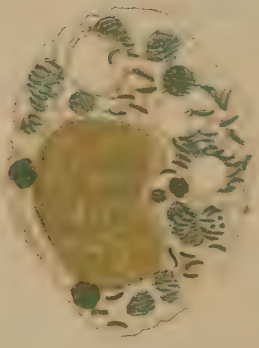

Fig. 35.—Macrophage of guinea-pig filled with cholera vibrios not transformed into granules.

[174] the phagocytosis becomes complete, and the organism gets rid of the vibrios solely by means of this reaction. Even seven hours after injection of the vibrios, when the peritoneal fluid, crammed with leucocytes, has become thick and turbid, there still remain a few scattered vibrios which always retain their shape and their normal activity. A drop of this exudation, maintained at 38° C. outside the organism, gives, in a few hours, an abundant culture of very active vibrios. It must, therefore, be concluded that the fluid part of the exudation was powerless to destroy the vibrios or even to render them motionless, whilst the living leucocytes have shown themselves capable of ingesting and digesting them. The peritoneal exudation, withdrawn at a period when it no longer contains any free vibrios, still gives cultures of the organism for some time. Soon, however, there comes a period when the inoculated exudation remains sterile, this proving that the vibrios, ingested in a living state by the phagocytes, have at length been killed by the microphages and macrophages.

When, instead of cholera vibrios of medium virulence, we take a variety completely deprived of pathogenic activity, it is sometimes observed that certain of these organisms, when injected into the peritoneal cavity of the normal guinea-pig, become transformed into spherical granules in the fluid of the exudation without any direct [175] co-operation of the phagocytes. This transformation into granules was first studied by R. Pfeiffer[1] and hence has been termed Pfeiffer's phenomenon. It is of limited occurrence in natural immunity and is produced, as I have been able to demonstrate, only under certain well defined conditions. Pfeiffer's phenomenon is observed in the peritoneal fluid. It commences soon after the injection of the vibrios and takes place during the period of phagolysis. In other parts of the body of the guinea-pig, notably in the subcutaneous tissue and in the anterior chamber of the eye, Pfeiffer's phenomenon does not manifest itself; the animal, none the less, resists the inoculation of the vibrios. Even in the peritoneal cavity, moreover, it is easy to check the granular transformation of the vibrios by means which prevent the production of phagolysis. When we inject into the peritoneal cavity of a guinea-pig a foreign fluid, capable of exciting the phagocytic action, e.g. veal broth, physiological salt solution, urine, etc., we first excite a transitory phagolysis. To this stage succeeds another in which the leucocytes become very numerous and much more resistant than before. If we take advantage of this period of leucocytic stimulation to inject vibrios which have been attenuated as much as possible, we shall observe that they soon become the prey of the peritoneal phagocytes, without manifesting any sign whatever of Pfeiffer's phenomenon.

It is evident, then, that this extracellular destruction of the vibrios, sometimes observed in the peritoneal cavity, is really the work of the microcytase that has escaped from the phagocytes during their period of transient injury.

Having analysed the mechanism of natural immunity against certain bacilli, spirilla and vibrios, it will be interesting to determine whether the same rules are to be applied in the case of the cocci. Choice is not difficult since we may equally well fix upon the staphylococci, the pneumococci, streptococci or gonococci. Should we decide upon the streptococcus it is solely because the natural immunity against this micro-organism has attracted the special attention of

[1] *Ztschr. f. Hyg.*, Leipzig, 1894, Bd. XVIII, S. 1.

several observers. A second advantage of the streptococcus, however, is the high degree of natural immunity manifested against it by
[176] a laboratory animal so convenient as the guinea-pig. Dr Jules Bordet[1] studied this subject in my laboratory. He observed that the injection of streptococci into the peritoneal cavity sets up a marked leucocytosis which ends in a complete destruction of the micro-organisms. The leucocytes rapidly ingest the great majority of the streptococci and destroy them; there remain only a few isolated and free individuals which are protected by a clear zone (aureola) which develops around them, but in the end they also become the victims of the voracity of the phagocytes. When we increase the dose of streptococci injected, phagocytosis still goes on, but some of the streptococci succeed in escaping, and we see a new generation produced which is distinguished by the thickness of the protective aureola. In spite of the afflux of a large number of leucocytes, they no longer ingest the streptococci and generalisation of the infection results, followed by the death of the animal. Natural immunity, then, can be suppressed under certain definite conditions. Dr Jules Bordet[2] wished to satisfy himself whether the leucocytes failed to fulfil their phagocytic function because of the paralysis of their movements, or as the result of some other weakness. With this object he injected into the peritoneal cavity of guinea-pigs, at the moment when the streptococci begin to get the upper hand of the leucocytes, a definite quantity of a culture of *Proteus vulgaris*. These small bacilli in a short time become the prey of phagocytes which, however, still refuse to ingest streptococci (fig. 36). There is thus in the peritoneal cavity a kind of selective process as regards the ingestion of these microbes. The *Proteus* disappears as the result of phagocytosis, whilst the streptococci thrive in the fluid of the exudation

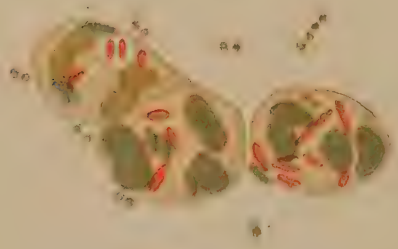

Fig. 36.—Peritoneal exudation from guinea-pig showing free streptococci and microphages that have ingested *Proteus* bacilli.

[1] *Ann. de l'Inst. Pasteur*, Paris, 1897, t. xi, p. 177.
[2] *Ann. de l'Inst. Pasteur*, Paris, 1896, t. x, p. 104.

and continue to multiply. This experiment, which readily succeeds, demonstrates very clearly the difference between the positive sus- [177]
ceptibility of the leucocytes (with respect to the *Proteus*) and the negative (with respect to the streptococcus). Bordet, in accordance with the view now generally accepted, regards this sensitiveness as a chemiotaxis, that is to say a perception of the chemical composition of the surrounding medium. It must be admitted that the substance which excites the chemiotaxis of the leucocytes does not readily diffuse and may not, therefore, be found in a state of solution in the plasma of the peritoneal exudation. Otherwise the leucocytes would refuse to ingest, not only the streptococci, but also the small *Proteus* bacilli, bathed in the same repellent fluid. It is more probable that the substance which excites the negative chemiotaxis is contained in the aureola that surrounds the streptococci, from which it only escapes with difficulty and for a short distance.

Marchand[1] continued the investigation of the same subject in Denys' laboratory at Louvain. He studied the natural resistance of the guinea-pig, rabbit and dog against the streptococcus. He, also, came to the conclusion that phagocytosis constitutes the principal means of defence of these mammals in their struggle against one of the most formidable of the pathogenic micro-organisms. Starting from a single colony, Marchand obtained two distinct races, one very virulent for the rabbit, the other encountering a most effective natural resistance. This resistance is due to the activity of the phagocytes which destroy the streptococci in the ordinary fashion. He states as the general result of his investigation that "an attenuated streptococcus is a streptococcus readily devoured by phagocytes" whilst "a very virulent streptococcus is a microbe that is not attacked by the leucocytes," and he adds that "a streptococcus is virulent because it is not devoured by phagocytes" (*l.c.* p. 270). Up to this point the views of Marchand are in accord with those of Bordet; but here they diverge, in fact as soon as it becomes a question of the explanation of the origin of the difference in the behaviour of the leucocytes. Marchand refuses to apply the theory of chemiotaxis and asserts "that the phagocytosis depends on some physical property of the streptococcus and is consequently dependent on the tactile functions of the leucocytes" (p. 292). The experiments upon which he founds his conclusion cannot, however,

[1] *Arch. de méd. expér. et d'anat. path.*, Paris, 1898, t. x, p. 253.

be regarded as absolutely demonstrative. Thus, Marchand observed
[178] that the attenuated streptococci, when conveyed in the culture-fluid of the virulent variety, are as readily devoured by the phagocytes as when they were injected alone. According to him, therefore, there was in the culture-fluid of the virulent streptococcus no soluble substance capable of exciting the negative chemiotaxis of the leucocytes. But is it quite proved that this substance must necessarily pass into the filtrate of a virulent culture? If it adheres closely to the glairy aureola, as we have suggested, may it not remain behind with the bodies of the streptococci, without passing through the filter in any appreciable amount? The question cannot be regarded as definitely settled, but probability appears to be on the side of the theory of chemiotaxis.

Marchand also investigated whether the immunity against the attenuated streptococcus might not be explained by the bactericidal activity of the fluids of refractory animals. His results were unvarying and definite. The blood serum of his animals never exhibited any bactericidal power against the streptococcus, and the attenuated race, like the virulent one, grew well in the serums of the rabbit, dog and guinea-pig.

More recently, Wallgren[1] has taken up the study of the immunity and susceptibility of rabbits with respect to the streptococcus. His conclusions are, on the whole, in accord with those of his predecessors. He found that if the injected streptococci were not very virulent phagocytosis began immediately after the injection into the peritoneal cavity and continued as long as there were any streptococci to be attacked. In those cases, on the other hand, where the streptococcus was endowed with a greater virulence, a transitory phagocytosis took place at the beginning of the infection; but the streptococci soon succeeded in adapting themselves to the struggle with the leucocytes and kept them at a distance. The multiplication of the streptococci could then go on without restraint and the animal soon succumbed to a generalised infection. Wallgren considers that, in the defence of the organism against the streptococcus, the products of the destroyed leucocytes may, sometimes, play a part.

As the mechanism of natural immunity against the groups of bacteria—bacilli, spirilla (and vibrios) and cocci—presents a very great analogy in all three, it might be considered superfluous to continue

[1] *Ziegler's Beitr. z. path. Anat.*, Jena, 1899, Bd. xxv, S. 206.

our analysis of this phenomenon. Our review, however, would be incomplete if we omitted to take note of the natural immunity of the [179] animal organism against micro-organisms which are distinguished by an exceptional toxicity. The first place in this group must undoubtedly be assigned to the bacillus of tetanus.

It may appear very inconsequent to be told that animals very susceptible to tetanus, such as the guinea-pig and rabbit, are endowed with a natural immunity against the tetanus bacillus. And yet this fact, paradoxical as it may seem, has been demonstrated beyond doubt by Vaillard and his collaborators Vincent and Rouget[1]. When a small quantity of a culture of the tetanus bacillus was injected into one of the animals just mentioned, tetanus was not long in declaring itself. After a period of incubation, certain muscles became stiff and a tetanus, local at first, soon became general and had a fatal issue. Now, when much larger quantities of bacilli are inoculated, but care is taken to rid them of the tetanus poison elaborated in the culture-fluid, the animals resist without exhibiting any trace of tetanus. This experiment, repeated many times, always with the same result, demonstrates that the tetanus bacillus, when deprived of the co-operation of the toxin, encounters, in these animals so susceptible to the latter, a most effective opposition. Why is this? It was supposed that, in diseases like tetanus so markedly toxic in character, the resistance was in no way dependent on the phagocytic function. Thus Vaillard and Vincent were quite prepared to attribute no share to the phagocytes in the example of natural immunity which they had discovered. A detailed analysis of the facts convinced them, however, that in this they were in error. Guinea-pigs and rabbits do not contract tetanus, after the inoculation of a quantity of spores and bacilli of tetanus deprived of their toxin, [180]

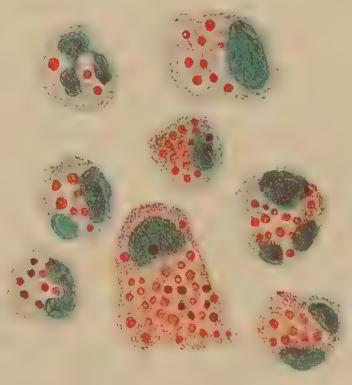

Fig. 37.—Leucocytes of rabbits filled with tetanus spores.

[1] *Ann. de l'Inst. Pasteur*, Paris, 1891, t. v, p. 1; 1892, t. vi, p. 385; 1893, t. vii, p. 755.

solely because of the occurrence of very pronounced phagocytosis. Such an injection is soon followed by a very marked invasion of leucocytes which cram themselves with spores and bacilli without being in any way inconvenienced thereby (Fig. 37). Once the phagocytes have devoured all these organisms, the latter become incapable of producing their morbific effect. The spores cannot germinate within the phagocytes, but there undergo a marked degeneration and finally, after a longer or shorter interval, disappear.

When, on the other hand, the tetanus bacilli or their spores are accompanied by the pre-formed toxin, the latter, according to Vaillard, excites a negative chemiotaxis of the leucocytes which keep away from the organisms and which are thus allowed to multiply and to secrete fresh quantities of toxin. The natural immunity of the animal's organism against the tetanus bacillus can be suppressed whenever the phagocytic defence is hampered in any way. Under natural conditions it is usually the adjuvant micro-organisms that aid the tetanus infection by hindering the phagocytes from seizing the spores with sufficient rapidity to prevent their germination. This fundamental result, established by Vaillard and Vincent, has often been gainsaid on the evidence of insufficient experiments (Sanchez-Toledo, Klipstein, Roncali), but, ultimately, its accuracy has been completely confirmed. Cases have been cited in which the tetanus spores, deprived of their toxin, still set up a fatal tetanus. When a small fragment of an agar culture of tetanus, previously heated to 85° C. for the purpose of destroying the toxin, is inoculated, we produce tetanus. Vaillard and Rouget have demonstrated that, under these conditions, the leucocytes penetrate merely into the superficial layer of the agar, the spores germinating and the bacilli multiplying in the deeper part. We can also set up a fatal tetanus in animals by inoculating, along with sterilised earth, spores deprived of their toxin by means of heat. The particles of soil protect the spores against the aggression of the phagocytes, allow them to germinate and then to poison the organism. Lactic acid produces an analogous effect, by destroying or weakening the phagocytes. Micro-organisms, most often inoffensive in themselves, also prevent the phagocytosis of the tetanus spores and thus aid the intoxication.

[181] The facts above summarised have been demonstrated to be the rule for several species of anaerobic pathogenic bacteria. Thus, Besson[1] showed that the septic vibrio is, by itself, incapable of setting

[1] *Ann. de l'Inst. Pasteur*, Paris, 1895, t. IX, p. 179.

up septicaemia; in order to do this it needs the co-operation of other micro-organisms. Leclainche and Vallée[1] have extended the same rule to the bacillus of symptomatic anthrax (*Bacillus chauvaei*), so important as being the cause of an epizootic disease of the Bovidae. The spores of this bacillus when heated to 80°—85° C. lose the preformed toxin and at once become incapable of producing infection. In this case also, these spores soon after injection become the prey of phagocytes, which seize them, prevent their germination and check their pathogenic action. If to these heated spores, however, we add a small quantity of toxin, they are enabled to germinate in the tissues and set up a typical infection. If heated spores are mixed with sterile sand, and the mixture introduced into guinea-pigs, these animals almost invariably acquire a fatal symptomatic anthrax. The spores in the superficial part of the sandy mass are readily devoured by the phagocytes; but those which are included within the central part of the mass, being protected for some time against these cells, germinate as soon as they become permeated with the fluids of the animal organism. If we envelope the sand in a paper sac the protection against the phagocytes is still more complete and allows almost all the spores to germinate and in every case to set up a fatal infection. Leclainche and Vallée conclude from their experiments "that we only require to protect the spore *mechanically* in order to see an infection produced; here we cannot allege an increase of its virulence, as when we associate a chemical substance with the virus, and the exclusive part played by the phagocytosis in the protective process stands out clearly" (p. 221).

The history of these three anaerobic organisms clearly proves that the natural immunity against them cannot be made dependent on either the bactericidal power of the fluids, or on any antitoxic property, or on the incapacity of the micro-organism to secrete its toxin in the fluids of the refractory animal. The cause of this immunity resolves itself into the reaction of the phagocytes which prevent the micro-organisms from producing their poisons.

All that has been said on the subject of the natural immunity of the Vertebrates has had reference to cases of resistance against [182] Bacteria. But may not the immunity against micro-organisms belonging to other groups depend on other factors with which the reader has not yet been made sufficiently acquainted? Amongst the lower plants there are Blastomycetes (*Torulae* and Yeasts) which are

[1] *Ann. de l'Inst. Pasteur*, Paris, 1900, t. XIV, p. 202.

capable of producing infections, *e.g.* the disease amongst the *Daphniae.*

Some observers, no doubt, have come to the conclusion that the various Blastomycetes, when introduced into a refractory organism, undergo complete destruction within a few hours without any intervention of phagocytosis. Thus Jona[1] explains the disappearance of yeast-cells injected into the veins or peritoneal cavity of the rabbit as due to the sole influence of the microbicidal property of the blood-fluid. Gilkinet[2] looks at it from the same point of view. He injected beer yeast (*Saccharomyces cerevisiae*) into a rabbit and observed that it had disappeared shortly afterwards. The destruction of the yeast-cells, according to this observer, "is effected by means of plasmatic juices" and "is due to a specific property of the organic fluids" whose nature is "quite unknown as regards its essential principle." Phagocytosis is said to play no part in this phenomenon. Let us hasten to say that before the publication of the two works just cited, a memoir by Schattenfroh[3] had appeared on the same subject. This observer, who carried out his experiments in Buchner's laboratory at Munich, accurately observed and described the destruction of injected yeasts by phagocytes, whilst his experiments on the microbicidal power of the blood and serum failed. This testimony is the more important that it emanates from a school by whom the microbicidal power of the "humours" is regarded as the principal factor in the defence of the animal organism. The facts described by Schattenfroh are perfectly accurate and have been confirmed in my laboratory by Skchiwan[4], who did not restrict himself to injecting ordinary yeasts (pink yeast, *Saccharomyces pastorianus*) but inoculated guinea-pigs with pathogenic yeast-cells, isolated by Curtis[5] from a case of
[183] myxomatous tumour in man. The guinea-pig is refractory to small doses of this yeast but succumbs to injections of larger quantities: Skchiwan convinced himself that the ingestion of the non-pathogenic yeast-cells takes place with great rapidity. Thus the *Saccharomyces pastorianus*, in the peritoneal cavity of the guinea-pig, is ingested almost exclusively by microphages at the end of two hours. Some (3—4) hours after injection, "sowings" of the peritoneal exudation

[1] *Centralbl. f. Bacteriol. u. Parasitenk.*, Jena, 1897, Bd. XXI, S. 147.
[2] *Arch. de méd. expér. et d'anat. path.*, Paris, 1897, t. IX, p. 881.
[3] *Arch. f. Hyg.*, München u. Leipzig, 1896, Bd. XXVII, S. 234.
[4] *Ann. de l'Inst. Pasteur*, Paris, 1899, t. XIII, p. 770.
[5] *Ibid.*, 1896, t. X, p. 448.

no longer yield growths. On the other hand Curtis' pathogenic yeast-cells resist the action of the phagocytes for a much longer time. After a period of phagolysis in the peritoneal cavity, the leucocytes that have just arrived in large numbers begin to seize the yeast-cells. Usually several macrophages fuse around the same yeast globule forming a very characteristic kind of rosette. Sometimes the macrophages run together to produce a giant cell, whose centre contains the yeast-cell. This latter defends itself against phagocytosis by secreting a fairly thick membrane. The struggle between the two living elements is a fairly prolonged one; 24 to 48 hours after inoculation all the yeasts are surrounded by phagocytes, amongst which microphages are exceptional. But the parasites remain alive for 4—6 days after their injection into the peritoneal cavity, as proved by the cultures that are obtained from the exudation when the fluid is "seeded" out. It must be concluded, therefore, that the yeast-cells were surrounded by the phagocytes whilst still presenting all the signs of life. Skchiwan was no more successful than Schattenfroh in demonstrating any kind of microbicidal action of the fluids on the Blastomycetes.

There is, consequently, no doubt whatever that the resistance of the animal organism against yeasts follows the same rules that hold in the defence against bacteria.

The animal micro-organisms are much rarer in infective diseases than are the microphytes; moreover the impossibility of obtaining cultures of them renders their investigation much more difficult. Yet there exist facts that are capable of affording us information as to the means made use of by the refractory organism against certain parasitic Protozoa. Amongst these latter the *Trypanosomae* play a most important part. One species of this genus (*T. lewisi*) produces an infective disease in rats, especially in the grey rat (*Mus decumanus*), the blood of these rodents often containing a very large number of them, whilst the small flagellated organisms flourish well in the serum prepared from the blood of affected animals. Laveran and [184] Mesnil[1], in their studies on the *Trypanosomae*, injected defibrinated blood containing numerous *Trypanosomae* into the peritoneal cavity of guinea-pigs, which exhibit a natural immunity against this parasite. The parasites remained alive for some days and then disappeared completely. Here again it is the phagocytes of the peritoneal exudation which rid the animal of the *Trypanosomae* by ingesting them. Laveran and Mesnil were able, by the examination of hanging drops

[1] *Ann. de l'Inst. Pasteur*, Paris, 1901, t. xv, p. 673.

of the peritoneal exudation of their guinea-pigs, to detect leucocytes in the act of devouring *Trypanosomae* which showed, by their active movements, that they were still alive. Once the parasites were completely enclosed within the macrophages, their final disappearance took place with extraordinary rapidity.

In this chapter we have attempted to place before the reader a complete series of the phenomena observed in natural immunity in animals. We have passed in review the resistance of the animal organism against the principal groups of bacteria, and we have dwelt on certain of them which are most capable of adapting themselves to various media, and on others which present examples of micro-organisms more exacting and more delicate. We have examined the immunity against Blastomycetes and parasitic animalcules. Above all, in the lower animals, just as in the Vertebrata of all classes, we have always observed this general phenomenon: phagocytic resistance as the principal and constant factor in natural immunity.

CHAPTER VII

THE MECHANISM OF NATURAL IMMUNITY AGAINST MICRO-ORGANISMS

The destruction of micro-organisms in natural immunity is an act of resorption.—Part played by inflammation in natural immunity.—Importance of microphages in immunity against micro-organisms.—Chemiotaxis of leucocytes and ingestion of micro-organisms.—Phagocytes are capable of ingesting living and virulent micro-organisms.—The digestion of micro-organisms in phagocytes is most often effected in a feebly acid medium.—Bactericidal property of serums.—Phagocytic origin of the bactericidal substance.—Theory of the secretion of the bactericidal substance by leucocytes.—Comparison of the bactericidal power of serums and of blood plasmas.—The bactericidal substance of blood serums must not be considered a secretion-product of leucocytes; it remains within the phagocytes, so long as they are intact.—The cytases.—Two kinds of cytases: macrocytase and microcytase.—Cytases are endo-enzymes, allied to trypsins.—Changes in the staining properties and in the form of micro-organisms in the phagocytes.—Absence or rarity of fixatives in the serums of animals endowed with natural immunity.—The agglutination of micro-organisms does not play any important part in the mechanism of natural immunity.—Absence of anti-toxic property of the body fluids in natural immunity.—The phagocytes destroy the micro-organisms without their ingestion being preceded by neutralisation of the toxins.

THE facts we have set forth in the preceding chapter clearly justify us in concluding that the destruction of the micro-organisms in natural immunity is reduced to their resorption by the phagocytes.

We have now, therefore, returned to the point arrived at and already studied in Chapter IV, where we attempted to establish certain fundamental laws. It remains to be seen up to what point these laws apply to the phenomena of natural immunity against infective micro-organisms.

The introduction into the animal organism of foreign blood, of spermatozoa belonging to the same or a different species, or of any other cells, as in the case of the penetration of micro-organisms into

the tissues or cavities of the body of a refractory animal, determines, primarily, a localised inflammation, associated with which is a diapedesis of many white corpuscles. Instead of aseptic inflammation, as
[186] in the case of the resorption of cells, there is produced, in antimicrobial immunity, a septic inflammation at the point of invasion of the micro-organisms. In this inflammation the redness and heat are slight, the fluid part of the exudation is insignificant, but what is especially characteristic is the large number of leucocytes which come up towards the point menaced. This constancy of the inflammatory reaction in natural immunity is one of the best proofs of the accuracy of the view that inflammation is a phenomenon useful to the animal organism, especially in its struggle against microbial invasion. As we have devoted a whole volume to the discussion of the comparative pathology of inflammation it is here unnecessary to discuss it further. Since the publication of this book numerous articles on inflammation have appeared, but none of them have undermined, in the least degree, the fundamental bases of the phagocytic theory of inflammation. The view that this phenomenon really constitutes a healing reaction of the organism is at present accepted by many investigators in all countries. It is therefore needless to re-defend it.

Although there still remain a certain number of points that are not sufficiently cleared up in the essential mechanism of inflammation, it is now proved beyond doubt that the sensitiveness of the cell elements which here play a part, is one of the essential factors in the process. The nerve cells which govern the vascular dilatation, the endothelial cells which allow of the passage of leucocytes, and the leucocytes themselves which escape from the vessels in order to reach the point of entrance of the micro-organisms, all must be influenced in a special fashion. In natural immunity the phagocytes exhibit a positive chemiotaxis and this form of sensitiveness is a condition indispensable to a state of immunity and to the disappearance of the micro-organisms.

In my eighth lecture on inflammation I have already set forth the fundamental facts upon which rests the doctrine of the chemiotaxis of leucocytes. During the last ten years numerous data corroborating these results, obtained first by Leber, Massart, and Charles Bordet, and since confirmed by numerous other observers, have been accumulated.

In the resorption of blood corpuscles and of animal cells in general, it is especially the macrophages which intervene, but in

natural immunity against micro-organisms positive chemiotaxis is exhibited by the microphages more than by the macrophages. [187] When we examine an inflammatory exudation and find a preponderance of microphages we are satisfied that there has been an intervention of micro-organisms. Even in the examples where it is, at first, principally the macrophages which destroy the micro-organisms (as in the case of the resistance of the animal organism against the tubercle bacillus), there is also a great afflux of microphages. The sensitiveness of the two chief categories of phagocytes often exhibits a marked difference. We need merely recall to the reader the example of the spirilla, ingested and destroyed exclusively by the macrophages of the guinea-pig, which alone exhibit the necessary positive chemiotaxis. In many other examples of natural immunity the part played by the macrophages is masked by that of the microphages.

In natural immunity the motile phagocytes, having come up to the invaders, perform a second physiological function ; they ingest the micro-organisms. Sometimes the leucocytes devour at one swoop whole masses of these organisms, and carry out their work in a very short time. In other cases, especially when actively motile micro-organisms, such as the spirilla of Obermeyer or of Sacharoff, have to be dealt with, the ingestion takes place with more difficulty and requires special conditions. Thus, in order to ingest a spirillum, the macrophages of the guinea-pig throw out long conical processes. Never in the ingestion of micro-organisms have I observed methods comparable to that by which the macrophages seize upon the red corpuscles of birds or upon other animal cells.

Some observers have expressed the opinion that micro-organisms make their way into the cells spontaneously and do not need to be drawn in by means of protoplasmic processes thrown out by the phagocytes. It is of course indisputable that certain micro-organisms may pass into the interior of the cell independently of any act of phagocytosis. Such is the case with the malaria parasite and allied species which make their way into the red blood corpuscles. But here we are dealing with amoeboid organisms, quite capable of perforating the wall of the red blood corpuscle by means of their own pseudopodia. Bacteria, which do not possess amoeboid movements, are deprived of this power of invasion. There are, however, very rare cases in which such penetration does take place. For [188] example, Bizzozero[1] has described spirilla in the stomach of the

[1] *Arch. f. mikr. Anat.*, Bonn, 1893, Bd. XLII, S. 146.

dog; these may be found inside epithelial cells. But here these actively motile bacteria make their way into the interior of vacuoles which open on the free surface. Attracted, probably, by the epithelial secretions the spirilla first draw near to the cells and then take advantage of small openings through which they pass into the secretory vacuole. In almost all cases, however, living and even actively motile bacteria are incapable of penetrating into cells. Thus, when we observe the spirilla of recurrent fever or of goose septicaemia in the neighbourhood of leucocytes, we often see them exhibit very brisk corkscrew movements on the surface of these cells without ever being able to invade them. On the other hand, when the leucocyte sends out a process towards the spirillum ingestion rapidly takes place. In anthrax exudations, or in the spleen of animals that have succumbed to anthrax, large numbers of bacilli may often be observed in the immediate neighbourhood of the leucocytes or of the cells of the splenic pulp, without a single bacillus being found within these cells. Nor do we ever see any bacteria (which develop abundantly in a drop of exudation withdrawn from the organism) invade the dead leucocytes, lying alongside them. Whilst on the other hand we see the micro-organisms swarming outside the neighbouring leucocytes and occupying the free spaces between these cells.

Almquist[1] has recently described a method by means of which micro-organisms can be taken into the substance of dead leucocytes. He collects leucocytes from mammalian blood, mixes them with bacteria, and centrifugalises the mixture for some time. He convinced himself that after a not very prolonged contact the bacteria are found within leucocytes. Here Almquist excluded phagocytosis, properly so-called, that is to say, the ingestion of the bacteria by the active movements of the leucocytes; but he does not give sufficient proof that the cells, in his experiments, were actually dead. He thinks that the relatively low temperature (below 15° C.) excluded the possibility of amoeboid movement in the leucocytes of warm-blooded
[189] animals. This argument, however, does not accord with actual fact, for it is indisputable—and we have often convinced ourselves of this—that the leucocytes of man and warm-blooded vertebrates maintained at even a lower temperature than 15° C. are quite capable of motion and of ingesting foreign bodies. In all cases, the data as a whole, some

[1] *Ztschr. f. Hyg.*, Leipzig, 1899, Bd. xxxi, S. 507. See review by Podwyssotsky in the *Arch. russes de Path.*, St Pétersb., 1899, t. viii, p. 257.

of which we have cited above, leave no doubt that the ingestion of micro-organisms unprovided with amoeboid powers takes place by means of active movements of the living protoplasm of the leucocytes. To dissipate any remaining doubt on the part of the reader I need only recall Bordet's investigations, cited in the preceding chapter, of the behaviour of leucocytes in the peritoneal cavity of guinea-pigs inoculated with streptococci and *Proteus* bacilli. The leucocytes of the peritoneal cavity allow the virulent streptococci to develop freely, not ingesting a single one, whilst the *Proteus* bacilli, injected later, are quickly devoured and at the end of a very short time are all found in the substance of these same phagocytes. This example, so demonstrative, of the chemiotaxis (positive as regards *Bacillus proteus* and negative as regards the streptococcus), is at the same time the best proof of the fact that the ingestion of the micro-organisms is a vital, physiological act and not merely a simple phenomenon of mechanical penetration of micro-organisms into the soft protoplasm of the leucocytes.

It was formerly thought that leucocytes, charged with micro-organisms, provide the latter with a good culture medium and serve also as vehicles of transport for them from one place to another in the living organism. This view has often been affirmed without any proof whatever being given of it. It has now been demonstrated to be erroneous. The micro-organisms, with some rare exceptions, find within the leucocytes a very unfavourable medium. Usually they perish there, or, in the case of very resistant micro-organisms, such as the tubercle bacilli in refractory animals or the endospores of certain bacteria, without being actually destroyed, they are prevented from germinating and multiplying.

Later, another view has been advanced that phagocytes are capable of ingesting only those micro-organisms that have been previously killed by some substance which is found outside the defensive cells. This view is quite as erroneous as the one we have just analysed. The phagocytes are perfectly capable of seizing and devouring living micro-organisms. We have only to recall on this point the facts cited in the preceding chapter on the subject of living [190] bacteria ingested by the leucocytes of various animals, or the history of the very active spirilla which retain their motility up to the moment when they become completely enclosed by the protoplasmic processes of the leucocytes of the guinea-pig. Observations *in vitro* have, as already described in the same chapter, afforded a demonstration of the

ingestion of living flagellated Infusoria by the leucocytes of refractory animals.

These facts, fairly numerous in themselves, are not, however, the only ones that might be cited in favour of the fundamental thesis that phagocytes possess all the means for incorporating living micro-organisms. In my first works on phagocytosis I cited the example of amoeboid cells, in the Invertebrata, containing motile bacteria[1], and that of leucocytes of the frog charged with motile bacilli[2] of an artificial septicaemia. Since then the number of similar cases has increased considerably. Nothing is easier than to observe the phagocytosis of living micro-organisms *in vitro*. Take a drop of frog's lymph and add to it a few of the *Bacilli pyocyanei*, we soon observe the struggle between the leucocytes and the very motile bacteria, and inside the digestive vacuoles bacilli executing very pronounced and active movements.

The same result may be obtained by another method, by which at the same time we gather information as to the virulence of the micro-organisms ingested by the phagocytes. The view has often been expressed that phagocytes seize only those bacteria that have been deprived of their virulence by a previous action of the fluids of the animal organism; consequently search has been made for some attenuating property of these fluids. We have already answered this objection in the previous chapter by the citation of cases in which the exudations of refractory animals, containing only micro-organisms ingested by the phagocytes, were, nevertheless, very virulent for susceptible animals. This question has been especially discussed in relation to the anthrax of frogs, on which subject several investigations have been carried out, the result of which is completely convincing. Bacilli ingested by the leucocytes of these Batrachians retain their full virulence for a long time. Exudations which contain only intra-
[191] phagocytic bacilli, the majority of which have already lost their normal staining by aniline dyes, produce fatal anthrax in susceptible animals, such as the mouse and the guinea-pig. Mesnil has demonstrated the same fact by using the exudations of fresh-water fishes that are refractory to anthrax. The same rule applies equally to the exudations of dogs and fowls that have been inoculated with the bacillus.

Long before these experiments on anthrax were made, Pasteur[3]

[1] *Arb. a. d. zool. Inst. d. Univ. Wien*, 1883, tom. v, S. 160.
[2] *Biol. Centralbl.*, Erlangen, 1883–4, Bd. III, S. 562.
[3] *Compt. rend. Acad. d. sc.*, Paris, 1880, t. XC, p. 952.

had shown that the virus of fowl cholera, which in the guinea-pig sets up a mild affection and gives rise to the formation of abscesses, retains its virulence for a considerable time in the pus of these abscesses. When he injected rabbits with a small quantity of guinea-pig's pus developed at the point of inoculation of the cocco-bacillus of fowl cholera, the animals succumbed to a generalised and rapid infection. The conviction has since been arrived at that, in the guinea-pig, these micro-organisms readily become the prey of the leucocytes that are present in the exudations.

The rule, therefore, is general that in animals endowed with natural immunity the phagocytes seize and ingest even living micro-organisms that have retained their initial virulence.

Once within the phagocytes, the micro-organisms are surrounded by a clear fluid, which accumulates in vacuoles, or they are lodged directly in the protoplasm. In both cases the micro-organisms are subjected to a digestive action which usually dissolves them completely. It is not always easy to form an idea of the conditions under which the intracellular digestion takes place. At first[1] I used a weak solution of vesuvin for the purpose of gaining some idea as to the condition of the micro-organisms that have been ingested by the leucocytes and demonstrated that the living bacteria remain unstained in this solution, whilst the dead bacteria take on a somewhat deep brown stain. Thanks to this reaction I was able to furnish one of the proofs of the fact that in immunised animals ingested bacteria are killed inside the phagocytes. The use of Ehrlich's neutral red (*Neutralroth*) gives us further valuable indications. This colour, quite innocuous for living elements, is an excellent indicator of acid or alkaline reaction. Plato[2], in Breslau, has carried out numerous [192] researches on the staining of micro-organisms by a weak aqueous solution ($1^0/_0$) of this substance. He has shown that "free" micro-organisms remain alive in this solution without taking on any tinge of colour. On the other hand, the same micro-organisms, when ingested by the phagocytes, are stained brownish-red. Most of these stained organisms no longer exhibit any sign of vitality; but amongst those within the phagocytes are some which, in spite of being deeply stained, are certainly alive. Plato insists on the fact that ingested micro-organisms remain stained as long as the phagocytes are alive, for, shortly after the death of these cells, decoloration of the

[1] *Ann. de l'Inst. Pasteur*, Paris, 1887, t. I, p. 325.
[2] *Arch. f. mikr. Anat.*, Bonn, 1900, Bd. LVI, S. 868.

micro-organisms and of the intracellular granules takes place. When neutral red is added to an exudation in which the leucocytes are dead, the staining of the ingested micro-organisms—dead or living—does not take place. I have myself verified these observations, and Himmel[1], who has carried out an elaborate investigation on this subject in my laboratory, has confirmed them in numerous cases. In the third and fourth chapters of this work I have already brought forward arguments in favour of the view that the staining of the ingested elements indicates a feebly acid reaction inside the phagocytes. Sometimes this reaction manifests itself in the digestive vacuoles; in other cases it is exhibited only in the micro-organisms directly lodged in the protoplasm (Fig. 38). Whilst the phagocyte is still living the acid juice which fills the vacuoles or permeates the ingested organisms does not mix with the protoplasm which is always alkaline. But shortly after the death of the phagocytes this mixture is effected without difficulty, and the alkalinity of the protoplasm is then amply sufficient to neutralise or even render alkaline the feebly acid juices. This interpretation of the facts is in complete harmony with all the data, collected up to the present, on the staining by neutral red of phagocytised micro-organisms.

Fig. 38.—Peritoneal macrophage of guinea-pig that has ingested a number [193] of *Bacilli coli*. Stained *intra vitam* with neutral red.

All ingested bacteria do not, however, stain in the way we have indicated. Tubercle bacilli, even in cases of natural immunity, remain unstained inside the phagocytes or take on only a very slight straw-yellow tint. Himmel made this observation on the bacilli of avian tuberculosis that had been ingested by the peritoneal leucocytes of the guinea-pig, a species resistant to this micro-organism. It might be thought that such a resistant membrane as that of the tubercle bacillus, with its waxy layer, would prevent the penetration of the acid leucocytic juice; but several bacilli which resist decoloration by acids, as do the tubercle bacilli, notably the bacilli

[1] *Ann. de l'Inst. Pasteur*, Paris, 1901, t. xv, p. 928.

of Moeller and their allies, are stained a bright red by neutral red as soon as they are ingested by the phagocytes. It is, therefore, more probable that, in the case of true tubercle bacilli, the reaction in the cells is no longer acid, but alkaline. This conclusion is confirmed by what is observed in the giant cells of the Algerian gerbil (*Meriones shawii*), a species of rodent which exhibits a great natural resistance against the bacillus of human tuberculosis[1]. The bacilli, ingested by these phagocytes, secrete a series of concentric membranes which become impregnated with phosphate of lime (Fig. 5). The process causes the death of the bacilli, of which there remain only the calcified membranes. The precipitation of the lime salt around bacillary membranes itself indicates the alkaline reaction of the medium. The use of certain staining substances fully confirms this conclusion. Thus, with alizarin sulpho-acid the giant cells stain deep violet, this affords clear proof of a very distinct alkaline reaction.

We arrive then at the general conclusion that phagocytic digestion usually takes place in a medium weakly acid, but that it can also go on in an alkaline medium. It is impossible, in the present state of our knowledge, to define the nature of the acid secreted by the phagocytes. H. Kossel[2] has expressed the view that the intracellular digestion of micro-organisms is effected by the nucleic acid, secreted [194] by the cell nucleus and accumulated in the vacuoles of the contents of the phagocytes. He has brought forward in support of this view the fact that nucleic acid is distinctly bactericidal, killing certain pathogenic micro-organisms, and giving a precipitate composed of albumen and nucleic acid. Later A. Kossel pointed out the presence in these formed elements of albuminoid substances which have an alkaline reaction but which also destroy bacteria. Thus he has isolated from the spermatic fluid of the sturgeon a protamine, "Sturin," which, even in very weak solutions, exhibits a strong bactericidal action on the typhoid bacillus, staphylococcus, etc. It is possible that these substances play a part in intracellular digestion. On the other hand, however, we must regard it as well established that in phagocytes there is a soluble ferment which kills and digests micro-organisms. We have already seen, in connection with the resorption of animal cells, that it is the ferment alexine, or cytase, which plays the principal part in the digestive function. We must

[1] "Leçons sur la pathologie comparée de l'Inflammation," Paris, 1892, p. 193; authorised English translation, London, 1893, p. 162.

[2] *Arch. f. Physiol.*, Leipzig, 1894, S. 200.

now ask ourselves whether the same substance acts also on micro-organisms.

For more than fifteen years a study of the bactericidal power of the blood and other fluids drawn from the animal organism has been carried on. Based on the not very definite results of Traube and Gscheidlen[1], Fodor[2] drew attention to the property of the defibrinated blood of the rabbit to destroy the bacteria sown in it. Under the inspiration of Flügge[3], Nuttall[4] carried out a whole series of experiments on this bactericidal property of defibrinated rabbit's blood, of the aqueous humour, and of some other fluids. After confirming Fodor's general result, Nuttall went further and showed that the bactericidal power of the fluids is due to a substance of undetermined nature which is destroyed by heating to 55° C. for one hour. This discovery was confirmed by a large number of observers, and soon became an accepted fact.

Flügge now considered that he could base a theory of immunity on the presence of the bactericidal substance of the body fluids. Bouchard[5] and his school adopted and developed this view, especially with reference to researches on the microbicidal power of blood
[195] serum. Buchner[6] soon came forward as the chief advocate of this theory, and enriched it by numerous investigations carried out by himself or along with collaborators in his school at Munich. It is to him that we owe the suggestion of the term *alexine* (protective substance) to designate the bactericidal substance of blood serum and other fluids of the animal organism which are capable of killing micro-organisms. Buchner determined the conditions under which alexine acts best as a bacterial poison and developed the humoral theory of natural immunity, according to which the latter is reduced to the bactericidal property of the body fluids.

As the postulates of this theory are often not in accord with the real facts, as Lubarsch[7], especially, has demonstrated in many of his

[1] *Jahresb. d. schles. Gesellsch. f. vaterl. Cultur*, Breslau, 1874.

[2] *Deutsche med. Wchnschr.*, Leipzig, 1886, S. 617; 1887, S. 745.

[3] *Ztschr. f. Hyg.*, Leipzig, 1888, Bd. IV, S. 208.

[4] *Ztschr. f. Hyg*, Leipzig, 1888, Bd. IV, S. 353.

[5] "Les microbes pathogènes," Paris, 1892.

[6] *Arch. f. Hyg.*, München u. Leipzig, 1890, Bd. 10, S. 84; *Centralbl. f. Bakteriol. u. Parasitenk.*, Jena, 1889, Bd. V, S. 817, and Bd. VI, SS. 1, 561; 1890, Bd. VIII, S. 65.

[7] *Centralbl. f. Bakteriol. u. Parasitenk.*, Jena, 1889, Bd. VI, S. 481; *Ztschr. f. klin. Med.*, Berlin, 1891, Bde XVIII, XIX.

papers, we[1] expressed the opinion that a portion at least of the bactericidal power might come from substances that had escaped from the leucocytes during the preparation of the defibrinated blood and of the blood serum. This hypothesis remained for several years unnoticed, but later several observers have, quite independently, arrived at the conclusion that alexine is nothing but a leucocytic product. Denys and Havet[2] were the first to show that exudations rich in white corpuscles exhibited a bactericidal power much higher than that of the corresponding blood serums. Shortly afterwards H. Buchner[3] showed the same thing on comparing the bactericidal power of exudations rich in leucocytes with the blood serum of the same animals. As this property disappeared from both fluids after they had been heated to 55° C., Buchner concluded that the bactericidal substance of the exudations must be identical with the alexine of the blood serum. Several other observers, amongst whom Bail, Schattenfroh, Jacob and Löwit, may be cited, obtained results more or less in accord with the above, though obtained by different methods, so that it has now for some time come to be recognised that the leucocytic origin of the alexines is generally accepted, especially since Jules Bordet[4], in an investigation carried out in my [196] laboratory, arrived at the same result from various very demonstrative experiments.

Nevertheless several authoritative voices have been raised against this interpretation of the facts. R. Pfeiffer especially, with his school, has pronounced against the leucocytic origin of the bactericidal substance found in the blood serum. Pfeiffer and Marx[5] and Moxter[6] have insisted on the fact that the fluids of exudations rich in leucocytes are often much less bactericidal than is the serum of the blood of the same animals.

For some years, struck by the marked difference between the phagocytic function of the macrophages and that of the microphages, I have thought that the contradictory results of the observers cited might be explained by some difference in the nature of the leucocytes of the various exudations and of the blood which served for the

[1] *Ann. de l'Inst. Pasteur*, Paris, 1889, t. III, p. 670.
[2] *La Cellule*, Lierre et Louvain, 1894, t. x, p. 7.
[3] *München. med. Wchnschr.*, 1894, S. 717.
[4] *Ann. de l'Inst. Pasteur*, Paris, 1895, t. IX, p. 462.
[5] *Ztschr. f. Hyg.*, Leipzig, 1898, Bd. XXVII, S. 272.
[6] *Deutsche med. Wchnschr.*, Leipzig, 1899, S. 687.

preparation of the serums. I therefore asked Gengou to devote his attention to this particular point and to compare the bactericidal power of exudations, rich in microphages, with that of others containing many macrophages and also with the blood serum of the same animals. Gengou[1] has carried out his experiments with remarkable exactness and care, and as I have followed them closely I am in a position to speak as to their extreme accuracy.

In order to obtain exudations very rich in microphages Gengou injected gluten-casein by Buchner's method into the pleural cavity of dogs and rabbits. Usually at the end of 24 hours he was able to collect a large quantity of fluid containing numerous leucocytes, almost exclusively microphages. To obtain macrophagic exudations Gengou injected washed red blood corpuscles of the guinea-pig into the pleural cavity of his animals; two days afterwards he withdrew from the pleural cavity a very viscid fluid, containing, as regards formed elements, macrophages almost exclusively. After isolation of the leucocytes by centrifugalisation of the exudations, Gengou washed the cells with physiological salt solution and then added to them an equal volume of broth. This mixture was frozen by Buchner's method, and was then submitted to a temperature of 37° C. Under
[197] these conditions the leucocytes, killed by cold, gave up to the fluid their bactericidal substance.

Studied in this way, the bactericidal power of the extract of microphages showed itself always superior to that of the corresponding blood serum. The greatest difference was observed in the dog, where, as already mentioned in the preceding chapter, the serum of the blood has no bactericidal property as regards the anthrax bacillus, whilst the extract of microphages manifests this property very strongly. The microphagic extract of the exudations of rabbits was more active in the destruction of the bacilli of anthrax and typhoid, *Bacillus coli* and the cholera vibrio, than was the blood serum.

The result of these experiments leaves no room for doubt. The microphages, collected in the aseptic exudations of the dog and rabbit, contain more bactericidal substance than does the blood serum of the same animals. Nor can there be a doubt that this bactericidal substance is the same whether it appears in the microphages or in the blood serum: in both cases it is destroyed by heating to 55° C. and, in all other respects, it behaves in the same manner.

[1] *Ann. de l'Inst. Pasteur*, Paris, 1901, t. xv, p. 68.

The experiments of Gengou with the extracts of macrophages have demonstrated, on the other hand, that this fluid exerts no bactericidal power. Let it be understood at the outset that this fact is in no way an indication of the absence of the bactericidal ferment in the macrophages. Direct examination of the phenomena which are manifested inside these cells demonstrates most clearly that the macrophages kill and digest micro-organisms. But this process usually goes on much more slowly in the macrophages than in the microphages, owing probably in the former to the presence of a smaller quantity of the bactericidal substance. Under these conditions we can readily understand that this substance does not pass, or passes only in small amount, into the extracts. There is nothing remarkable in the fact that, with so imperfect a method of preparing the extracts, the greater part of the bactericidal substance should remain in the bodies of the cells.

The facts just set forth afford a sufficient explanation of the marked difference in the results obtained by various observers as to the bactericidal power of the exudations. When the latter are rich in microphages, the bactericidal property is very marked: when, on the other hand, the exudations contain a large number of macrophages, the bactericidal power may be very weak or even *nil*.

The experiments above summarised confirm the conclusion that [198] the microphages must be regarded as the source of the bactericidal substance of the body fluids. But here arises the question: Do the microphages secrete the substance during life, giving it up to the blood plasma, or does this substance escape only after the death of the leucocytes and the damaging of the cells, due to various external causes? We here touch on a problem which has been the subject of much discussion and one of very great importance in connection with the question of Immunity in general.

After the discovery of the bactericidal power of serums, several investigators set to work in search of the source of the bactericidal substance. Hankin[1], and shortly afterwards Kanthack and Hardy[2], expressed the view that this substance is the secretion-product of the eosinophile leucocytes which would thus appear to be a kind of motile unicellular glands. This theory could not be supported by solid

[1] *Centralbl. f. Bakteriol. u. Parasitenk.*, Jena, 1892, Bd. XII, SS. 777, 809; 1893, Bd. XIV, S. 852.

[2] *Proc. Roy. Soc. London*, 1892, Vol. LII, p. 267; *Phil. Trans.*, London, 1894, (B) Vol. 185, pt. I, p. 279.

arguments and must be regarded as generally abandoned, because it is now completely out of accord with well-established facts. Thus, various osseous fishes, in spite of the total absence of eosinophile or pseudo-eosinophile granules are none the less capable, thanks to their leucocytes, of destroying a large number of pathogenic micro-organisms (Mesnil, *l. c.*).

A similar theory was enunciated by H. Buchner[1], though he holds that it is not the eosinophile leucocytes only that secrete the bactericidal substance, but the leucocytes in general. Being attracted to the point menaced by the micro-organisms, these cells secrete their bactericidal product, which diffuses into and along with the plasma of the exudations and of the blood. In these fluids the micro-organisms undergo a more or less complete destruction, or at least severe injury which renders them more susceptible to the attack of the phagocytes. At the International Congress of Hygiene, held at Budapest in 1894, Buchner proclaimed the thesis that "the leucocytes fulfil an important function in the natural defence of the organism...by means of soluble substances which they secrete." Later, his pupils, Hahn[2] and Schattenfroh[3], endeavoured to support
[199] this theory by exact experiments, but they found it impossible to do this at all satisfactorily. Later, another of Buchner's pupils, Laschtschenko[4], published a paper in which he maintains that he has found a convincing argument. It is as follows. A blood serum, by itself void of bactericidal property, some minutes after white corpuscles from another species of mammal have been added to it acquires this property. Thus the rabbit's leucocytes added to dog's serum immediately give to it the bactericidal power, so long as a large number of cells remain alive and motile. But when the leucocytes of the same species are added to rabbits' serum the fluid becomes no more bactericidal than before. The same result may be obtained by mixing rabbits' leucocytes with the blood serum of the horse, pig and other species. Laschtschenko concludes from these observations that the vital secretion of the bactericidal substance by the leucocytes of the rabbit takes place when they are irritated by the serum of a different species. As an analogous effect has been observed with mixtures of

[1] *München. med. Wchnschr.*, 1894, S. 717 and 1897, S. 1320.

[2] *Arch. f. Hyg.*, München u. Leipzig, 1895, Bd. XXV, S. 105; 1897, Bd. XXVIII, S. 312. *Berl. klin. Wchnschr.*, 1896, S. 864.

[3] *Arch. f. Hyg.*, München u. Leipzig., 1897, Bd. XXXI, p. 1; 1899, Bd. XXXV, S. 135. *München. med. Wchnschr.*, 1898, SS. 353, 1109.

[4] *Arch. f. Hyg.*, München u. Leipzig, 1900, Bd. XXXVII, S. 290.

rabbits' leucocytes with the serum of a different species heated to 60° C., Laschtschenko believes himself safe from the objection that the giving up of the bactericidal substance results from the death or injury of the white corpuscles. According to him this injurious effect on the white corpuscles can only be produced by an unstable substance which is destroyed by heating to 60° C. Laschtschenko forgets that the leucocytes are in general delicate cells, capable of being affected even by fluids which do not actually kill them. Now we know that serums, when heated to 60° C., still retain their power of agglutinating the leucocytes, a power which must hamper these cells in their normal function.

Trommsdorff[1], in an investigation carried out in Buchner's laboratory, endeavoured to supplement Laschtschenko's results and to support them by new and more convincing experiments. But he only succeeded in a few cases in obtaining a bactericidal serum after adding rabbits' leucocytes to the blood serum of other animals. "In a great number of my experiments," says Trommsdorff, "I very often did not succeed in extracting the alexines from the rabbit's leucocytes by the use of Laschtschenko's method" (p. 385). On the other hand, Trommsdorff, wishing to establish the living condition of the leucocytes mixed with a foreign serum, arrived at the following result: "In the majority of the cases, as in fresh exudations, the number of living leucocytes after their treat- [200]
ment with active horse's serum, as well as with inactive serum (heated to 60° C.) of dog, ox and horse, varied between 60 and 80 %" (p. 391). In spite of these verifications, Trommsdorff comes to the conclusion that the presence of alexine in those serums to which leucocytes had been added, must "in all probability" be attributed to its secretion by the living leucocytes. We regard it as much more probable that the alexine, in those cases where it passed into the serum, was due to the breaking up of the dead leucocytes, whose numbers rose to 40 %, that is to say, almost half their total number. Our conclusion is, in any case, much more in accord with the more constant and more exact results obtained by other methods.

In spite of the insufficiency of proofs in favour of the theory of bactericidal secretions by the leucocytes it has been very favourably received by many investigators. As, however, it came into collision with the general fact that, in the refractory animal, the micro-

[1] *Arch. f. Hyg.*, München u. Leipzig, 1901, Bd. XL, S. 382.

organisms remain alive in the plasmas of the exudations and are, in this condition, ingested by the phagocytes, it was therefore very important that this fundamental contradiction should be settled by decisive experiments. The attempt has often been made to obtain blood plasma and to compare its bactericidal action with that of serum from the same animal. In the preceding chapter we have already mentioned an attempt in this direction made by Sawtchenko. Hahn[1] had previously attempted to prepare plasma by adding histon to blood. As this "plasma" was found to be just as bactericidal as the blood serum Hahn concluded that the bactericidal substance, secreted by the living leucocytes, circulates in the living blood. In all the experiments carried out by this method it was impossible to avoid certain sources of error, and in my laboratory Gengou[2] undertook a new series of researches, endeavouring to obtain from blood a fluid resembling normal plasma as closely as possible. The method he employed has been described in detail in a memoir, on an anti-coagulating serum, which he published along with Bordet[3]. The blood was drawn into paraffined tubes and centrifugalised at once in other tubes whose walls were likewise covered with a layer of
[201] paraffin. The fluid thus prepared is certainly more allied to circulating plasma than is the blood serum obtained after the coagulation of the blood. Nevertheless, it is still far from being identical with true normal plasma; it still coagulates, though tardily. Gengou compared, in their bactericidal action, the blood serum and the serum, decanted after the tardy coagulation of the fluid analogous to plasma. He carried out a great number of experiments with the two fluids, obtained from dogs, rabbits and rats, making a comparative study of their bactericidal power as regards the anthrax bacillus, the typhoid bacillus, and the cholera vibrio. I have closely followed all these experiments and can confirm the results described by Gengou, namely, that the fluid, in this plasma serum, possesses an insignificant bactericidal power or none at all, whilst the blood serum almost always exhibits this property to a marked degree.

As a result of the researches just summarised it is no longer possible to maintain the theory of bactericidal secretions by leucocytes or by any other category of cells. The bactericidal substance

[1] *Arch. f. Hyg.*, München u. Leipzig, 1895, Bd. xxv, S. 105; *Berl. klin. Wchnschr.*, 1896, S. 864.

[2] *Ann. de l'Inst. Pasteur*, Paris, 1901, t. xv, p. 232.

[3] *Ibid.*, p. 129.

does not circulate in the blood plasma nor in that of the exudations, and this is a sufficient reason for refusing to it the title of a secretion-product. Its presence in the blood serum is due, like that of the fibrin-ferment, to the destruction or more or less grave injury of the phagocytes.

This fact, upon which we must insist most strongly, is in flat contradiction to the view recently formulated by Wassermann[1]. In a work devoted to natural immunity against micro-organisms, this author describes how he submits his animals (guinea-pigs) to the action of an anticytase (or anti-alexine) serum whose preparation, described in the fifth chapter of this work, offers no difficulties. Under the influence of this serum, the guinea-pigs, into the peritoneal cavity of which a strong dose of typhoid cocco-bacilli is inoculated, die from infection, whilst the control animals, inoculated in a similar manner, but which have received in addition some normal rabbit's serum, heated to 60° C., entirely resist the infection. Wassermann concludes that the first series of guinea-pigs succumbed because of the impossibility of fighting against the typhoid bacillus by means of the free cytase, this being neutralised by the anticytase serum. The fact pointed out by Wassermann is perfectly accurately stated and has been confirmed by Besredka[2], in an investigation carried out in my laboratory. Nevertheless, it is impossible to accept Wassermann's view as to the part played by [202] anticytase in his experiment. As clearly demonstrated by Besredka, the anticytase serum acts not merely by neutralising the bactericidal ferment, but also by its other properties, especially by one which prevents the stimulation of the phagocytes.

In the struggle of the guinea-pig's organism against a strong dose of typhoid cocco-bacilli (in Wassermann's experiments 40 times the lethal dose), the free cytase plays a part so infinitely small that even the injection into a guinea-pig of a large quantity of serum (3 c.c.) from a normal guinea-pig (containing much cytase) does not prevent the death of the animal. It is only the blood serum of other species (rabbit or ox) that is capable of protecting a guinea-pig against such a large quantity of typhoid bacilli.

Wassermann was in error in supposing that his experiment was a case of natural immunity. It comes entirely within the range of the phenomena of acquired immunity. In fact, the natural immunity of the guinea-pig is only exhibited against a dose 40 times less than

[1] *Deutsche med. Wchnschr.*, Leipzig, 1901, S. 4.
[2] *Ann. de l'Inst. Pasteur*, Paris, 1901, t. xv, p. 209.

that employed by Wassermann. Consequently the control guinea-pigs which received such a huge quantity of the typhoid cocco-bacilli, going beyond 40 times the limit of their natural immunity, require to be preserved from death by the injection of a large quantity of blood serum heated to 60° C. from the normal rabbit. This serum, deprived of its cytase, retains its other properties, by which the organism of the guinea-pig profits, especially exercising a stimulating action on the phagocytes of the guinea-pig. The immunity of Wassermann's control animals was, then, really an acquired immunity, the result of the introduction into their organism of the stimulating serum of the rabbit. For this reason an analysis of the work of this observer must be postponed until we treat of the phenomena of acquired immunity under the influence of normal serums.

We must, then, persist in the opinion that the plasmas of the normal animal, containing no cytases, cannot play a bactericidal part in natural immunity, a part which devolves upon the cytase contained within the phagocytes.

This result accords well, also, with the whole of the facts bearing on the destruction of micro-organisms in the animal body. The transformation into granules of the attenuated cholera vibrios that is sometimes observed in the peritoneal cavity during the period of phagolysis, and the absence of this transformation under conditions where the peritoneal leucocytes are protected against this injury, is [203] clearly explained. In the first case, Pfeiffer's phenomenon is set up by the bactericidal substance which has escaped from the leucocytes that have been altered by the foreign substances injected into the peritoneal cavity; in the second case, this phenomenon is not produced because the leucocytes remain intact. The absence of this granular transformation in the anterior chamber of the eye and in the subcutaneous tissue is also readily explained by the fact that the bactericidal substance, not being present in the blood plasma, cannot pass into the exudations of the eye and subcutaneous tissue[1].

[1] Since Nuttall's first paper appeared a certain bactericidal action of the aqueous humour has been observed. This fact should be taken into consideration in the study of the question of the phagocytic origin of the bactericidal substance of the body fluids. If this substance really comes from the phagocytes, it should not be found in the transparent aqueous humour that contains no, or almost no, leucocytes. Now this fluid sometimes destroys a certain number of micro-organisms. This apparent contradiction is explained by the fact that the bactericidal action may be exercised by all kinds of fluids, such as physiological salt solution, nutritive broths, etc. The bactericidal property of the aqueous humour comes into this category. Its action is,

The bactericidal substance, then, is essentially some substance which remains inside the uninjured phagocytes in the living animal but which escapes from these cells when they are injured, either in the body of the animal or outside in the blood withdrawn from the organism. Buchner has given to this substance the name of alexine and we must now determine whether this substance is the same cytase which digests the formed elements on their resorption.

Since his first researches on the power of one normal blood serum [204]
to dissolve the red corpuscles of another species, Buchner[1] has maintained the identity of the haemolytic substance with the bactericidal substance of the same serum. In both cases we have to do, according to him, with one and the same substance of an albuminoid nature, with the same "alexine." In his later work, Buchner attempted to confirm and develop this thesis. Bordet[2] has, on several occasions, brought forward arguments in favour of the same view; but against this Ehrlich and Morgenroth[3] have declared themselves. According to these observers a single serum may contain several alexines or "complements." The same serum may even contain two complements, one of which is destroyed by heating to 55° C., whilst the other, much more stable as to the action of heat, resists this temperature. In one of their most recent memoirs, Ehrlich

as a rule, much more feeble than the action of serums and exudations and is not modified by heating to 55°—56° C. In certain aqueous humours, a little cytase, or true bactericidal substance, may come into play, for we find aqueous humours which coagulate and which, when centrifugalised, show a small deposit of leucocytes. These results have been obtained by Mme. Metchnikoff.

It must not be forgotten also that, even in the bactericidal action of blood serums, a certain factor is the change of medium which the micro-organisms experience with the plasmolytic phenomena which follow. But it is not possible to ascribe to this factor the whole of the bactericidal property of serums and exudations, as is done by Baumgarten (*Arb. a. d. pathol.-anat. Inst. zu Tübingen*, 1899, Bd. III, S. 1, and *Berl. klin. Wchnschr.*, 1900, SS. 136, 162, 192), and his pupils Jetter and Walz supported by A. Fischer (*Ztschr. f. Hyg.*, Leipzig, 1900, Bd. XXXV, S. 1). The idea of reducing the destruction of bacteria in serums and exudations to the effect of osmotic pressure has been recently elaborately analysed by v. Lingelsheim (*Ztschr. f. Hyg.*, Leipzig, 1901, Bd. XXXVII, S. 131). With great justness he comes to the conclusion that "the existence in extravascular blood or in serum, of bactericidal substances acting as soluble ferments can now no longer be denied" (p. 167). In studying this question we must not lose sight of the fact that these bactericidal substances (alexines, complements, or cytases) give rise to the production in the animal organism of antagonistic substances as described by us in the 5th Chapter.

[1] *Verhandl. d. Congresses f. inn. Med.*, Wiesbaden, 1892, S. 273.

[2] *Ann. de l'Inst. Pasteur*, Paris, 1900, t. XIV, p. 257; 1901, t. XV, p. 312.

[3] *Berl. klin. Wchnschr.*, 1900, SS. 453, 677.

and Morgenroth lay special stress on the importance of an experiment which has enabled them, by means of filtration, to separate two complements from the normal serum of the goat, one of them attacking the red corpuscles of the guinea-pig, the other those of the rabbit.

Max Neisser[1] has adopted this view of the plurality of alexines. According to Ehrlich and Morgenroth, the same serum may possess several complements which attack the red blood corpuscles of various species and other complements which attack micro-organisms. In favour of this thesis Neisser gives a summary of his experiments on the absorption of complements which, in his opinion, prove the plurality of alexines. By centrifugalising rabbit's blood serum to which he had previously added a certain number of anthrax bacilli, he obtained a fluid which no longer destroyed this bacillus but which still dissolved the red corpuscles of goat and sheep. There are then, according to Neisser, in the normal serum of the rabbit, at least two different complements ; one for the bacilli and one for the red corpuscles.

With the object of explaining the discrepancy between these results and those of his previous experiments, Bordet[2] undertook
[205] a new series of researches on the absorption of cytases. He first made it clear that the normal red corpuscles, when plunged into a normal haemolytic serum, are incapable of fixing all the cytase. When such a serum is centrifugalised, after a prolonged contact with red corpuscles of a different species, the fluid no longer dissolves normal red corpuscles. But if these latter be sensibilised by means of a specific fixative, the red corpuscles are dissolved in large numbers. It must be admitted that in this experiment we have to do with a single cytase because, before centrifugalisation, as after it, the red corpuscles of the same species are added. In the first case, however, these corpuscles were normal, whilst in the second they were sensibilised by the fixative.

When, after the first part of this experiment, that is to say, after the fixation of a certain quantity of cytase by the red corpuscles, we centrifugalise the mixture and add, not the sensibilised red corpuscles of the same species but the normal red corpuscles of a different species, we find that the latter still dissolve and fix a certain quantity of cytase. As the first experiment (with sensibilised red corpuscles)

[1] *Deutsche med. Wchnschr.*, Leipzig, 1900, S. 790.
[2] *Ann. de l'Inst. Pasteur*, Paris, 1901, t. xv, p. 303.

has shown that the whole of the cytase has not been absorbed by the red corpuscles, we readily understand that the portion remaining in the fluid will act on the normal red corpuscles of another species.

But when we fix the cytase to the sensibilised red corpuscles the absorption becomes complete and the addition of other species of red corpuscles no longer produces any solution. It is easy, therefore, by means of sensibilised red corpuscles, to take out the whole of the cytase from a serum. When to such a serum, thus deprived of the whole of its haemolytic cytase, we add bacteria, these latter show no sign of disintegration ; whilst previously, that is before the absorption of the cytase by the sensibilised red corpuscles, the same serum was highly bactericidal. Let us take a concrete example so that the reader may form some definite idea of the phenomena observed. Take a normal rat's serum which, in a very short time, transforms cholera vibrios into granules or deforms and dissolves anthrax bacilli. The same serum dissolves the red corpuscles of a different species. We will first leave this serum in contact with these red corpuscles sensibilised by the specific fixative. After the solution of a quantity of these red corpuscles, let us add to the serum a few cholera vibrios or anthrax bacilli. The vibrios, in this serum, are no longer transformed into granules and the anthrax bacilli undergo no change at all ; they stain in the normal fashion by basic aniline dyes, they [206] present neither deformations nor solution of their contents. In other words, no bactericidal action takes place in a serum that is deprived of its cytase by sensibilised red corpuscles.

Is it necessary to conclude from this and other analogous experiments that the cytase, fixed by the sensibilised formed elements (red blood corpuscles or micro-organisms), is always one and the same cytase? May it not be that these elements, impregnated with specific fixatives, become so greedy for cytases that it is easy for them to absorb not only one variety but several species of cytases?

The facts we have summarised in Chapter IV concerning the cytases, indicate that very probably there exist two kinds of cytases, connected with the two great groups of phagocytes. Extracts of the mesenteric glands, of the omentum and of the exudations, which are composed for the most part of microphages, do not dissolve the red corpuscles, but are, on the other hand, specially bactericidal. Sarassewitch has carried out numerous experiments on this point in my laboratory and has brought forward a large number of data

in favour of this theory of two phagocytic cytases. He found that, even when specific fixative is added to the extract of microphagic exudations (of rabbit), the sensibilised red corpuscles are not dissolved. It must then be accepted that microcytase, so active against bacteria, is entirely powerless against animal cells.

As the microphages seize, though rarely, and digest red blood corpuscles, spermatozoa and other cells of animal origin, it must be admitted that they also contain a small quantity of macrocytase, or that the microcytase, given time, is capable of dissolving these elements. On the other hand, the macrophages, in spite of their marked predilection for animal cells, also ingest and digest certain bacteria. This is due perhaps to the presence of a little microcytase or to the power that the macrocytase has of attacking micro-organisms. These questions are too subtle to be definitely resolved at present.

The duality of the cytases does not clash with the experiments of Bordet summarised above. We have only to admit that the formed
[207] elements, once they are impregnated with specific fixatives, become capable of absorbing not only the cytase which digests them, but also another which, without dissolving them, is simply fixed to them. Here we should have a phenomenon analogous to the fixation by fibrin of diastases, other than trypsin and pepsin, or to the fixation by silk threads of all kinds of soluble ferments.

It may be accepted, then, that the phagocytes elaborate two cytases: macrocytase, active for animal cells, and microcytase, which digests bacteria. This result up to a certain point has been anticipated by Schattenfroh's[1] experiments and foreseen by Max Neisser (*l. c.*).

It has already been noted that the reaction inside the phagocytes is usually feebly or very feebly acid, and only rarely distinctly alkaline. On the other hand, it is well known that cytases, in serums, act in an alkaline medium. It is certain therefore that these soluble ferments can carry on the process of digestion under varied conditions. Hegeler[2], working in Buchner's laboratory, has studied the influence of the alkalinity and acidity of the medium on the bactericidal action of serum. He comes to the conclusion that the destruction of micro-organisms can take place in a serum to which has been added small quantities of alkali (carbonate of soda) and also in a weakly acid serum (from the addition of small quantities

[1] *Arch. f. Hyg.*, München u. Leipzig, 1899, Bd. xxxv, S. 199.
[2] *Arch. f. Hyg.*, München u. Leipzig, 1901, Bd. xl, S. 375.

of sulphuric acid). Once the serum becomes distinctly acid the bactericidal power disappears at once.

Our knowledge of the cytases, as a whole, leads us to approximate these diastases to the group of trypsins, papain, amoebodiastase and actinodiastase. The cytases are elaborated by the phagocytes, but are not secreted into the plasmas and they remain inside the cells so long as these cells remain uninjured.

In this respect the cytases must be placed in the group of the "Endo-enzymes," according to the nomenclature of Hahn and Geret[1]. These observers have carefully studied the proteolytic diastase of the yeast of beer which likewise acts inside the cells without ever being excreted. This diastase, to which they give the name of "yeast endotrypsin" (Hefeendotrypsin), presents in general an undeniable relationship with the phagocytic cytases, from which it is distinguished [208] however by a greater sensitiveness to alkalis. Kutscher[2] in his researches on autodigestion in yeast has established analogous facts.

The cytases and endotrypsin are consequently endo-enzymes, as are also amoebodiastase, actinodiastase, plasmase (fibrin ferment) and the zymase of E. Buchner. All remain confined within the cells which have manufactured them and are not secreted or excreted, as are the sucrase and invertin produced by yeasts or Mucedinae.

Our present knowledge on the cytases is as yet far from perfect, which is not astonishing, seeing how recently the question has been brought forward. The cytases found in the serum of the same animal are the same, for we have seen that the macrocytase which dissolves red blood corpuscles is the same which digests spermatozoa; whilst the same microcytase digests bacilli, spirilla, and cocci. But in the serums of different species, the cytases differ. Thus the cytases of the dog are not the same as are those found in the serums of the rabbit or horse. Whilst the majority of the cytases are very sensitive to heat and are destroyed at a temperature of 55°—56° C., some, *e.g.* the microcytase of rat's serum, resist this temperature and are only destroyed at 65° C., presenting, consequently, an example of cytase stable to heat similar to that discovered by Ehrlich and Morgenroth.

It is as yet very difficult to establish whether, besides the cytases, there exist other endo-enzymes within phagocytes, that is to say, soluble ferments which do not pass into the serums on the destruction

[1] *Ztschr. f. Biol.*, München u. Berlin, 1900, Bd. XL, S. 117.
[2] *Sitzungsb. d. naturforsch. Gesellsch. zu Marburg*, 1900.

of the phagocytes, but continue within these cells. Our present methods of investigation do not enable us to come to any conclusion on this point. We know only that the digestion of the formed elements is more complete inside the phagocytes than in the serums. Thus, as we have seen in Chapter IV, the best spermotoxic and haemolytic serums never digest either spermatozoa or the nuclei of the red corpuscles of birds. And yet these elements are completely dissolved in the phagocytic contents. Does this difference depend on the fact that, in the serums, we get only a very small part of the
[209] macrocytase, or upon the injurious influence of the alkalinity of the serums on the macrocytase which acts better in weakly acid media, or on the presence in the phagocytes of other endo-enzymes still unknown? These are questions to which at present no definite answer can be given.

Just as animal cells, when ingested by phagocytes during resorption (see Chap. IV), immediately become permeable to stains, so in natural immunity do micro-organisms taken into phagocytes acquire the same property. Very often, under the influence of the phagocytic action, the ingested micro-organisms become stainable by eosin (fig. 36). This eosinophile transformation has been observed in the cholera vibrio, the anthrax bacillus and in *Proteus vulgaris*. It is probably widely diffused among the phagocytised bacteria. This fact demonstrates clearly that at least some of the eosinophile granules are derived from foreign bodies ingested by the phagocytes. Others of these granules are probably the result of the transformation of soluble substances absorbed by the phagocytes. In fact, during inflammation, many microphages which contain no foreign solid body, may often be seen charged with a quantity of small pseudo-eosinophile granules.

Certain vibrios and bacilli, when ingested by microphages, become transformed, almost immediately, into spherical granules. The cholera vibrio undergoes the same transformation in the peritoneal exudation at the moment of phagolysis, as also in blood serum. The *Bacillus coli*, the typhoid bacillus, and certain other cocco-bacilli do not change in the least, or change very slightly in serum, but exhibit the transformation into granules when inside microphages. The macrophages, on the other hand, digest the same bacteria (vibrios and cocco-bacilli) without these bacteria presenting any signs of this change of form. The bacterial membrane resists the influence of the phagocytic digestion longer than do the contents, but is in the long run also completely digested. After the ingestion and destruction of micro-organisms

by the phagocytes, débris of indeterminate form may, for long, be found in the cells, but I have never been able to demonstrate any solid excreta from them. We must suppose, then, that the undigested portions are not thrown out from the phagocytes.

When describing the solution of red blood corpuscles by normal serums, we have mentioned Ehrlich and Morgenroth's view that the cytases are incapable of fixing themselves to these cells without the help of fixatives. They cite in support of their opinion several examples of fixatives (intermediary substances or "Zwischenkörper") discovered by them in the serums of various species of mammals. Is this so with microcytase in respect to micro-organisms? If this soluble ferment is incapable alone of fixing itself upon the bodies of these parasites, the help of fixatives would be indispensable to it. The bactericidal property of the microcytase, then, would depend on the existence of another body (fixative) which, perhaps, might not owe its origin to phagocytes. The problem, then, has a wide general range. [210]

In one of his memoirs, Bordet[1] had already raised the question of the existence of this sensibilising (or fixative) property in normal serums. By mixing two normal serums coming from different species, he was sometimes able to demonstrate the existence of such fixatives. Thus the cholera vibrios, which do not undergo granular transformation in either the normal serum of the horse (which is capable only of arresting their movements and agglutinating them into a mass) or in that of the normal guinea-pig, readily become transformed into granules when placed in contact with a mixture of the two serums. Bordet, however, refrains from any hasty generalisation on this observation and proposes to make fresh researches on this subject. Independently, Moxter[2] has attempted to demonstrate the presence of fixative in the normal serum of the guinea-pig. When deprived of cytases by heat, this serum is incapable of transforming the cholera vibrios into granules; but when fluid from the peritoneal exudation of the same guinea-pig is added, the transformation takes place very rapidly. Nevertheless, as this exudation was already, by itself, capable of producing Pfeiffer's phenomenon, Moxter's conclusions on the presence of the fixative in the normal guinea-pig's serum cannot be accepted without a fuller analysis of the facts, and this demands fresh researches.

[1] *Ann. de l'Inst. Pasteur*, Paris, 1899, t. XIII, p. 295.

[2] *Centralbl. f. Bakteriol. u. Parasitenk.*, Jena, 1899, I^te^ Abt., Bd. XXVI, S. 344.

A recent investigation, carried out by Bordet[1] in collaboration with Gengou, devoted to the study of the absorption of cytases by micro-organisms that have been sensibilised by means of fixatives, also gives us information on the question which now occupies
[211] us. It was easy to demonstrate the presence of fixative in the serums in the case of the cholera vibrio and its allies, by reason of their transformation into granules, appreciable on microscopical examination. When a serum, which of itself is incapable of setting up this transformation, produces it directly we add another serum heated to 55° C., we must conclude that the latter fluid contains the cholera fixative, whilst the former contains only cytases. But, as the majority of bacteria do not undergo any analogous transformation in serums, we are, in these cases, without any criterion as to the presence of fixative. Bordet and Gengou have eliminated this inconvenience in determining the fixation of alexine by bacteria which undergo neither granular transformation nor any other visible change. A normal unheated serum, which always contains a sufficient quantity of cytases, is mixed with any micro-organism, *e.g.* with the anthrax bacillus or the cocco-bacillus of plague. The serum, decanted after a prolonged contact with these bacteria, remains quite as capable of dissolving the red corpuscles of a determined foreign species as it was originally. This proves that cytases remain in the serum and that they have not been absorbed by the bacteria. Repeat the same experiment with this difference, that instead of normal anthrax bacilli or plague cocco-bacilli we introduce into the unheated normal serum these bacteria after they have been sensibilised by the corresponding fixatives (that is to say, previously submitted to the influence of specific serums heated to 55° C.). After contact for a certain length of time with these bacteria the serum is no longer capable of dissolving the red corpuscles of a determined foreign species, thus demonstrating that the cytases have, thanks to the help of the fixatives, been linked to the bacteria. We see, therefore, that it is easy to determine whether a serum, whose properties are unknown, contains fixatives or not. It is heated to 55° C. and mixed with normal unheated serum to which bacteria are added. If, after contact with these latter the normal serum has lost the power of dissolving the red corpuscles (which it was capable of dissolving previously), it is because its cytases, thanks to the fixative which must be present in the heated

[1] *Ann. de l'Inst. Pasteur*, Paris, 1901, t. xv, p. 289.

serum, have been absorbed by the bacteria. In the other case, we conclude the non-existence of the fixative.

In their researches, Bordet and Gengou often employed normal unheated serums to which they added several species of bacteria. They demonstrated that in these mixtures the cytases remained [212] intact or nearly so. These soluble ferments were scarcely, if at all, absorbed by the bacteria, which proves that in the normal serums there are no fixatives in any appreciable quantity. Of all their experiments the one that interests us most was carried out with *Proteus vulgaris*. This organism placed in prolonged contact with normal guinea-pig's serum showed itself incapable of absorbing or fixing anything beyond the most minute quantities of the cytases. There is consequently no fixative for *Proteus* in normal guinea-pig's serum, or, if any exists, it is only in negligible quantity. And yet this same *Proteus vulgaris*, when injected into guinea-pigs, was in a short time ingested and destroyed by the phagocytes which assure to the animal a natural immunity of the most stable character. The facility with which the leucocytes of the guinea-pig devour the *Proteus* follows, among others, from an experiment by Bordet[1] carried out with quite another object. A guinea-pig, very ill as the result of the injection into its peritoneal cavity of a very virulent streptococcus, contained in the peritoneal exudation a quantity of empty microphages incapable of ingesting these streptococci. At this critical moment there was injected into the same position a mass of *Proteus vulgaris*. "At the end of a very short time, it is seen that the leucocytes which energetically refuse to ingest streptococci greedily seize upon the new organism offered to them ; and at the end of half-an-hour the whole of these organisms are found inside phagocytes."

Here, then, we have an actual proof of the fact that the phagocytes, in order to rid the animal organism of a microbe and assure to it a natural immunity, have no need of any previous help from an extraphagocytic fixative. The phagocytes act, so to speak, *motu proprio*, and themselves bring about the resorption of the intruders. The question of fixatives in normal serums, then, loses its importance for us and their origin no longer presents any essential interest for the problem with which we are at present occupied.

Can we conclude, from the data just summarised, that the cytases,

[1] *Ann. de l'Inst. Pasteur*, Paris, 1896, t. x, p. 107.

which in several respects approximate to the trypsins, have this further feature in common with them that they can act without the help of any fixative? It is known, as mentioned in Chapter III, that trypsin can digest alone, or in collaboration with enterokynase, that ferment of the intestinal juice which acts as such a powerful ad-
[213] juvant to the pancreatic ferments. Is this also the case with the cytases? The fact that when *Proteus vulgaris* is placed in contact with normal unheated guinea-pig's serum, it is incapable of absorbing cytases, although it is so readily digested by phagocytes, indicates rather that, for the fixation of cytases, the help of the fixative is indispensable. But, as this fixative is absent from the serum, and since, nevertheless, it must exist for the needs of digestion, it must clearly be concluded that it is found inside the phagocytes. Its quantity is perhaps so small that when it has passed into the serum its action is entirely lost or nearly so. Fresh researches are necessary to elucidate this delicate point.

But perhaps the phagocytes which, as we have just seen, can engage in a struggle with and ingest the micro-organisms without the latter being previously modified by the fixative, may be incapable of fulfilling their functions without the help of some other substance circulating in the blood plasma? Amongst these substances is one which manifestly acts upon the micro-organisms by rendering them motionless and agglomerating them into masses. This agglutinative property is met with in the normal fluids of many species of animals and is exercised upon many bacteria. It may be demonstrated not only in the blood serum, but also in the fluids of transudations and exudations and in certain secretions such as milk, tears, and urine. Little is known as yet of the mechanism of this agglutinative action, and we can the more readily refrain from entering into details concerning it as it is of no great importance from the point of view of natural immunity.

In the preceding chapter we have already spoken of the ingestion of cholera vibrios in the peritoneal cavity of guinea-pigs. In those cases in which the animals exhibit an effective resistance, the phagocytes devour the vibrios whilst they still exhibit very active movements. Even when a large majority are already seized by the leucocytes and only a few isolated free vibrios remain, these latter still continue to exhibit normal movements. These facts, repeatedly observed, clearly demonstrate that phagocytosis may take place without any previous agglutinative action; this does not, however,

prevent the micro-organisms, when united into motionless masses, from being ingested by the leucocytes with greater ease.

In the case of the typhoid bacillus, one of the most active of bacteria, the same facts may be observed as in the case of the cholera vibrio. [214] In animals that remain unaffected we often see the last free bacilli moving about actively between the leucocytes filled with microbes. In many other examples of natural immunity we constantly meet with phagocytes containing but a single or a small number of micro-organisms (streptococci, yeasts, etc.).

The presence of motile micro-organisms inside phagocytes proves also that it is possible for these cells to do without the help of agglutinative substance in carrying on their protective work. The most carefully studied case of the relations between natural immunity and agglutination is that met with in the anthrax bacillus. We owe it to Gengou[1], who at the Liège Bacteriological Institute carried out a very detailed investigation on this question. He showed that the bacillus of Pasteur's first anthrax vaccine is agglutinated by the blood serum of a great number of animals. But he also showed that the serums which have the greatest agglutinative action on this bacillus do not come from the most refractory species. Human serum agglutinates most strongly the bacillus of the first vaccine (in the proportion of one part of serum to 500 parts of culture) but man is far from being exempt from anthrax. Pigeon's serum, on the other hand, is completely without any agglutinative power, although this species resists not only the first vaccine but very often even virulent anthrax. The serum of the ox, a species susceptible to anthrax, is more agglutinative (1 : 120) than that of the refractory dog (1 : 100). There are, however, exceptional cases in which the agglutinative property corresponds to the degree of susceptibility. Thus the serum of the mouse has not the slightest agglutinative action on the bacillus of the first vaccine. But alongside this example is that of the rat, a species of moderate susceptibility to anthrax, whose serum possesses the least agglutinating power of all, acting only in the proportion of 1 : 10. All these facts fully justify the conclusion formulated by Gengou that "we cannot establish any relation between the agglutinating power and the refractory state of the animals to anthrax" (p. 319). This conclusion may be extended to the phenomena of the

[1] *Arch. internat. de Pharmacodyn.*, Gand et Paris, 1899, t. VI, p. 299; *Ann. de l'Inst. Pasteur*, Paris, 1899, t. XIII, p. 642.

agglutination of micro-organisms and to those of natural immunity in general.

Amongst the properties of humours, there exists one which might play a part in natural immunity against micro-organisms. I mean the
[215] power possessed by the blood and certain other fluids of the animal body to neutralise the action of microbial poisons. Perhaps, it may be suggested, the phagocytes are not capable of commencing to do their work except after a previous action of antitoxins. After the neutralisation of the principal means possessed by the micro-organisms to injure the organism, these parasites, having been rendered innocuous, might be readily destroyed by the phagocytic cells. We have already had occasion to treat this fundamental question. Thus, we have insisted in the preceding chapters on the absence of any parallelism between immunity against micro-organisms and that against their toxins, taking as our examples anaerobic bacteria (tetanus bacillus, septic vibrio, bacillus of symptomatic anthrax) in connection with which phagocytosis takes place without any help from the antitoxic function.

We must now pass directly to the examination of the question of antitoxins in the fluids of animals naturally refractory to the micro-organisms and of the ultimate part played by them in this immunity.

Examples of the presence of antitoxic serum in normal animals are very rare. It might be supposed that animals endowed with natural immunity against micro-organisms and at the same time against their toxins, present an appreciable natural antitoxic power. Let us examine some of the more typical examples. The fowl enjoys a very marked immunity against the tetanus bacillus and its toxin ; its blood and its serum, however, as demonstrated by Vaillard[1], exhibit no antitoxic power ; this observation has been confirmed by several other workers. The rat is very refractory to diphtheria ; it resists subcutaneous inoculation of a large quantity of diphtheria bacilli and vigorously withstands diphtheria toxin when injected anywhere but into the brain. It has been demonstrated by Kuprianow[2], in an investigation carried out under Loeffler's direction, that the blood serum and the emulsion of the organs of the grey rat (*Mus decumanus*) possess no antitoxic property. This fact has been con-

[1] *Compt. rend. Soc. de biol.*, Paris, 1891, p. 464.
[2] *Centralbl. f. Bakteriol. u. Parasitenk.*, Jena, 1894, Bd. XVI, S. 415.

firmed by other observers. Von Behring[1], in a review of the phenomena of immunity in general, sums up this question as follows: "we find no antitoxin in the blood of individuals that are naturally refractory." There are, however, certain exceptions, perhaps only [216] apparent, to this rule. Thus Wassermann[2] has shown that blood serum from healthy human beings is sometimes antitoxic to the diphtheria poison. The individuals who furnished this antitoxin maintained that they had never suffered from diphtheria. We know, however, that this disease is sometimes present in so benign a form that it may pass unnoticed. More conclusive appears the example of normal horses whose blood serum, as demonstrated by Meade Bolton[3] and Cobbett[4], is very often antitoxic for the diphtheria toxin. This property, however, is not found in every horse; in certain individuals it is entirely absent. This last fact affords an indication that the antitoxic property in the blood of horses has been acquired as the result of some affection produced by a bacillus allied to the diphtheria bacillus. This view has not yet been sufficiently examined and consequently cannot claim to be accepted as settled. Recently, Max Neisser and Wechsberg[5] have discovered an antitoxin in human blood which is capable of preventing the solution of the red corpuscles by the toxin of staphylococci. This antitoxic power varies considerably in different individuals and is probably to be accounted for by the fact that the staphylococcus is one of the most widely diffused organisms among the bacterial flora of the human body. The small lesions produced by these micro-organisms (acne, boils, etc.) are so frequent in man that they may readily bring about the production of an antitoxin. In this case, however, we have again an example of acquired antitoxic power.

The examples just summarised can in no way affect the general thesis that the phagocytes, in order to fulfil their microbicidal function in an animal endowed with natural immunity, have no need of any previous action of the body fluids to neutralise the corresponding toxins.

[1] Article "Immunität" in the 3rd edition of Eulenburg's *Real-Encyclopädie*, Wien, 1896.

[2] *Deutsche med. Wchnschr.*, Leipzig, 1894, S. 120 (of Vereins-Beilage).

[3] [*Journ. Exper. Med.*, New York, 1896, Vol. I, p. 543.]

[4] [*Journ. Path. and Bacteriol.*, Edin. and London, 1896, Vol. III, p. 328; *Lancet*, London, 1899, Vol. II, p. 332; *Centralbl. f. Bakteriol. u. Parasitenk.*, Jena, 1899, I^te^ Abt., Bd. XXVI, S. 548.]

[5] *Ztschr. f. Hyg.*, Leipzig, 1901, Bd. XXXVI, S. 299.

The facts and views analysed in these two chapters afford us a general picture of the phenomena exhibited in natural immunity against micro-organisms. The dominant feature is represented by the phagocytic reaction that is observed throughout the animal series and that is exercised against parasites belonging to all the microbial groups. Phagocytosis is exhibited not only by the macro-
[217] phages but also, in a high degree, by the microphages which stand out as the defensive cells *par excellence* against micro-organisms. Their action is divided into a series of vital physiological acts, such as sensitiveness to the micro-organisms and their products, amoeboid movements which serve to ingest the micro-organisms, and into chemical and physico-chemical processes, such as the destruction and digestion of the devoured organisms.

The phagocytes enter into a struggle against the micro-organisms and rid the animal organism of them without requiring any previous help on the part of the body fluids. Phagocytosis, exercised against living and virulent micro-organisms, is sufficient to ensure natural immunity. The bactericidal power of the serum, which for a long time served as the basis for a humoral theory of immunity, represents merely an artificial property, developed in consequence of the setting free of the microcytase of the leucocytes that have become disintegrated after the blood has been drawn. The agglutinative power of the normal fluids of the body plays no important part in natural immunity.

The phagocytes, in order to fulfil their function, can attack micro-organisms that are capable of producing toxins. Any anti-toxic action against these bacterial poisons is in no way necessary to allow of phagocytosis coming into action.

Taken as a whole, the data collected on natural immunity against micro-organisms clearly demonstrate that the destruction of these parasites in the refractory animal organism represents merely a special phase of the resorption of formed elements.

CHAPTER VIII

SURVEY OF THE FACTS BEARING ON ACQUIRED IMMUNITY AGAINST MICRO-ORGANISMS

The discovery of attenuated viruses and its application to vaccination against infective diseases.—Vaccination by microbial products.—Vaccination with serums.—The acquired immunity of the frog against pyocyanic disease.—The acquired immunity against vibrios.—Extracellular destruction of the cholera vibrio.—Part played by two substances in Pfeiffer's phenomenon.—Specificity of fixatives.—Phagolysis and its relation to the extracellular destruction of vibrios.—Part played by phagocytosis in the acquired immunity against vibrios.—Fate of the spirilla of recurrent fever in the organism of immunised guinea-pigs.—Acquired immunity against the bacteria of typhoid fever and pyocyanic disease.—Acquired immunity against swine erysipelas and anthrax.—Acquired immunity against the streptococcus.—The acquired immunity of rats against *Trypanosoma*.

Certain of the hypotheses on acquired immunity are of as ancient origin as are those on natural immunity. For example, it has for long been known that man is constitutionally refractory to certain diseases which are very fatal to cattle. It has also been recognised that after a first attack of a contagious disease, such as small-pox, measles, scarlatina, typhoid fever, etc., man acquires a lasting immunity; and that the same rule applies to domestic animals, for example, cattle that have recovered from cattle plague or sheep that have got better from sheep-pox, become refractory against these diseases.

The discoveries of variolisation and vaccination, as methods of conferring on man a resistance to small-pox, have notably advanced our knowledge upon acquired immunity. The researches on the properties of vaccine had already led to some important results. But it is only since the publication of Pasteur's investigation, carried out with his collaborators Chamberland and Roux, in the first place, and with Thuillier later, that we have been able to take up the study of acquired immunity in a really scientific manner. The first in this

[219] series of discoveries, which have opened up a path so fruitful to science and medical art, was the discovery of the attenuation of micro-organisms. The small cocco-bacillus of fowl-cholera after several weeks' culture in broth was found to have become markedly attenuated in virulence. To Pasteur the idea occurred of testing whether fowls that had resisted the inoculation of these attenuated organisms had acquired any real immunity against virulent fowl-cholera. Experiment confirmed his expectation and led to the discovery of the vaccine against this disease. The method was at once applied to other infective epizootic diseases and shortly afterwards Pasteur, Chamberland and Roux found a method of preserving sheep and cattle from anthrax. To attain this end it was found necessary to prevent the bacillus from producing spores (in this they succeeded by cultivating it in broth at a temperature of 42·5° C.), because these spores fix the virulence and prevent attenuation. Having overcome this main obstacle, Pasteur and his collaborators found that their cultures, thus deprived of spores, become attenuated on exposure to the air and so become transformed into vaccines. They were thus able to prepare their two anthrax vaccines which soon found such wide application in practice. A few years later, Pasteur and Thuillier discovered the vaccines against swine erysipelas and, in collaboration with Roux and Grancher, Pasteur made the first application of his discoveries to the vaccination of man against rabies.

The path thus opened up was traversed by many other investigators and led to many very remarkable discoveries. Vaccination with micro-organisms became a recognised method and in the hands of Arloing, Cornevin and Thomas, soon found its application to symptomatic anthrax. The next step in this onward progress of science was taken when Salmon and Smith, working at hog-cholera, demonstrated the possibility of vaccinating not only with hog-cholera bacilli, but also with culture fluids in which these organisms had developed. These fluids, when completely deprived of micro-organisms by filtration, protected the experimental animals from virulent hog-cholera. This discovery, at first sceptically received, was soon confirmed and extended by the work of other investigators. Beumer and Peiper extended it to the experimental disease set up by the typhoid bacillus in small laboratory animals; Charrin applied it to the disease that he produced by means of the bacillus of blue
[220] pus; and Chamberland and Roux prepared vaccines from the soluble

products of the septic vibrio and of the bacillus of symptomatic anthrax. And now, as the result of these investigations, vaccinations by microbial products are in everyday use in all research laboratories. The vaccinations now used (anthrax, symptomatic anthrax, swine erysipelas and rabies) are still being carried out by means of the inoculation of living viruses.

The comparative history of acquired immunity is still very incomplete. The facts known concerning the adaptation of unicellular organisms to all kinds of injurious influences of a physical or chemical nature enable us to perceive that acquired immunity is just as general in living beings as is natural immunity; but it is impossible, in the present state of our knowledge, to confirm this hypothesis by exact and experimental data. The reason for this lies in the great difficulty we have in carrying out experiments on the lower animals. The majority of the Invertebrata in captivity do not remain alive long enough and can not be sufficiently often inoculated for us to obtain in them a well marked acquired immunity against micro-organisms. Kowalevsky[1], the celebrated Russian zoologist, has tried to overcome these various difficulties by making use of Myriapods. He found first that *Scolopendrae*, when inoculated with anthrax bacilli, die therefrom during the heats of summer, the blood containing a number of anthrax bacilli. But when the temperature does not exceed 17°—18° C., a fairly large number of these myriapods survive. The same survival was observed when Pasteur's first vaccine was injected. Kowalevsky utilised the *Scolopendrae* that had resisted the first injection of anthrax bacilli to ascertain whether they had contracted an acquired immunity. The results were not absolutely demonstrative and Kowalevsky sums up his results in the following words: "I cannot say, therefore, that I have succeeded in solving this question of vaccination, but it appears to me very probable" (p. 607).

In view of this doubt, I asked Mesnil to make a fresh attempt, making use of *Scolopendrae* and inoculating them with anthrax bacilli. These creatures, however, were so delicate and so little capable of remaining alive under the artificial conditions of their captivity, that the attempt soon had to be abandoned. I tried to obtain better results with the larvae of *Oryctes nasicornis*; here [221] again the difficulties were too great. These insects exhibit a perfect natural immunity against certain micro-organisms, but for others they showed an insurmountable susceptibility. It is very

[1] *Arch. de zool. expér.*, Paris, 1895, 3e série, t. III, p. 591.

evident, then, that it is not an easy matter to set up an acquired immunity in the Invertebrata.

It was necessary, therefore, to go higher up the animal scale and have recourse to cold-blooded vertebrates. The choice naturally fell on the frog. I asked Dr Gheorghiewski[1], who was working in my laboratory, to try to vaccinate the Batrachians against pyocyanic disease. I ought first to state that the bacillus of blue pus is pathogenic for the frog, which it kills both at the ordinary laboratory temperature, and at that of the incubator, 30°—37° C. In the first case the fatal dose is much greater than in the second, but it is always easy to induce a fatal infection. In this respect, therefore, the *Bacillus pyocyaneus* is much better adapted for study than the anthrax bacillus or many other micro-organisms. Gheorghiewski vaccinated green frogs (*Rana esculenta*), which had been accustomed to the incubator temperature, 30° C., by injecting every 4 to 7 days considerable doses of cultures of *Bacillus pyocyaneus* heated to 80° C. in order to kill all the micro-organisms. Some (3—4) weeks afterwards the prepared frogs became more resistant to the *Bacillus pyocyaneus* than were the controls placed under the same conditions. The frogs, inoculated with a fatal dose of the bacilli, clearly exhibited a certain, though slight, degree of acquired immunity. They withstood a dose that was always fatal to the controls or even a dose and a half, but died when injected with double the fatal dose. The lymphatic fluid of the vaccinated frogs feebly agglutinated (1 : 20—1 : 30) the *Bacillus pyocyaneus* although it still formed an excellent culture medium for this organism. Gheorghiewski satisfied himself that the agglutination was insufficient to assure immunity to the frog. The bacilli agglutinated into clumps were very virulent.

A detailed examination of the phenomena observed in the immunised frogs revealed the following facts. During the earliest stage the bacilli, injected into the dorsal lymphatic sac, were found free in the fluid, retained their form and were not transformed into granules. The bacilli carried by the lymphatic current spread rapidly
[222] throughout the body. Very shortly after inoculation, however, some of the leucocytes began to ingest the bacilli which became transformed into spherules within these cells. Later, the phagocytic reaction increased and at the end of 15 to 20 hours all the bacilli were found inside leucocytes. It was easy to demonstrate that these

[1] *Ann. de l'Inst. Pasteur*, Paris, 1899, t. XIII, p. 314.

bacilli had been ingested in a living condition. Forty-eight hours after inoculation, no bacilli were to be found in the lymph of the dorsal sac, either inside or outside the cells. But this fluid when sown on nutrient media gave colonies of the *Bacillus pyocyaneus* up to 15 and even 18 days after inoculation.

We may conclude from these facts that the cold-blooded vertebrata are capable of acquiring immunity to a slight degree and that, in this acquired immunity, a marked phagocytosis may be observed, but no bactericidal action of the fluids.

In order to gain a more complete idea of the mechanism of acquired immunity, it is necessary to observe it in higher vertebrates in which a well developed immunity of this type is readily obtained. Here we must have recourse to mammals and pass in review an ample number of examples, before we attempt to give to our readers a general summary of the question.

For long, researches on acquired immunity were confined almost exclusively to the analysis of the facts observed in animals submitted to anti-anthrax vaccinations by means of the two vaccines of Pasteur. A large number of important facts were thus collected, the more weighty of which must be presented to the reader. But, before entering on the subject, a general orientation on acquired immunity in laboratory animals against vibrios is indispensable as this example dominates, so to speak, the whole of the chapter on acquired immunity against micro-organisms.

Von Behring and Nissen[1], in their researches on the bactericidal power of serums, examined, amongst others, several specimens of serums coming from animals that had been vaccinated against various micro-organisms. In the majority of the examples given by them the acquired immunity produced no increase in this power, but the blood serum of guinea-pigs that had been immunised against Gamaleia's vibrio (*Vibrio metchnikovi*) was found to be much more bactericidal as regards [223]
this micro-organism than the serum of normal susceptible guinea-pigs. These authors came to the conclusion that in acquired immunity, at least as regards the vibrio mentioned, the chief part is played by a bactericidal substance which is developed in the fluids of the vaccinated animals. They were content with the mere demonstration of this fact without making any attempt to follow the course of events in the destruction of the vibrios as it occurs in the organism of the vaccinated

[1] *Ztschr. f. Hyg.*, Leipzig, 1890, Bd. VIII, S. 412.

guinea-pig. R. Pfeiffer[1] in collaboration with Issaeff sought to fill this gap. But, instead of taking Gamaleia's vibrio, these observers concentrated their attention on the study of the acquired immunity of guinea-pigs against the cholera vibrio. As this vibrio is as a rule less virulent than Gamaleia's vibrio, it was necessary, in order to obtain a fatal infection, to inject it, not into the subcutaneous tissue but into the peritoneal cavity. We have already seen (Chapter VI) that the cholera vibrio when inoculated into the peritoneal cavity of the guinea-pig, there meets with a vigorous resistance on the part of the leucocytes which seize the living and virulent vibrios and digesting them rid the animal of their presence. But when the dose of the vibrios is increased, they multiply in spite of the phagocytic reaction; they are found swarming in the peritoneal cavity, whence they invade the lymphatic and blood vessels and cause the death of the animal. It is easy, then, to induce a fatal infection of the guinea-pig with the cholera vibrio. But it is also easy to vaccinate these animals against this experimental disease. We have only to inoculate them with a non-fatal quantity of living cholera vibrios, or to inject into them a culture in which the vibrios have been killed by heat, or some of the culture fluid from which the vibrios have been removed by filtration. All these methods soon produce an acquired immunity in guinea-pigs. If, when this has been brought about, a little blood is withdrawn and to the serum a small quantity of cholera vibrios is added, *in vitro*, we can readily demonstrate their disappearance, under the influence of the bactericidal substance dissolved in the fluid. In this respect there is, then, a marked analogy with the fact established by v. Behring and Nissen as regards Gamaleia's vibrio.

When into the peritoneal cavity of vaccinated guinea-pigs a certain quantity of cholera culture containing virulent and very
[224] motile vibrios is injected, we find that in the peritoneal fluid drawn off by means of a fine pipette, the vibrios have undergone profound changes in the refractory organism. Even a few minutes after the injection of the vibrios, the leucocytes disappear almost completely from the peritoneal fluid; and only a few small lymphocytes and a large number of vibrios, the majority of which are already transformed into granules, are found (fig. 39); and there is presented a most typical case of Pfeiffer's phenomenon. Alongside

[1] *Ztschr. f. Hyg.*, Leipzig, 1894, Bd. XVII, S. 355, and *Deutsche med. Wchnschr.*, Leipzig, 1896, SS. 97, 119.

the round granules may be seen swollen vibrios, and others which have kept their normal form, but all are absolutely motionless. Some of these granules are gathered into small clumps, others remain isolated in the fluid. When to the hanging drop containing these transformed vibrios a small quantity of a dilute aqueous solution of methylene blue is added, we observe that certain granules stain very deeply, whilst others take on merely a very pale tint, scarcely visible. Many of these granules are still alive, because it is easy to watch them develop outside the animal and elongate into new vibrios. A large number of the granules, however, no longer exhibit any sign of life and are evidently dead. R. Pfeiffer and certain other observers affirm that the granules may be completely dissolved in the peritoneal fluid just as a piece of sugar dissolves in water. We have repeatedly sought for this disappearance of the granules in hanging drops of the peritoneal fluid, without being able to find any diminution in the number of these transformed vibrios, even after several days; nor have we been able to observe the phenomenon of the solution of the granules. It is at any rate indisputable that this granular transformation is a manifestation of very profound lesions undergone by the cholera vibrios under the influence of the peritoneal fluid of the immunised animal.

Fig. 39. Cholera vibrios in the peritoneal cavity showing Pfeiffer's phenomenon.

An attempt has been made to define the mechanism of Pfeiffer's phenomenon more exactly, and Fischer[1] has sought to refer it to osmotic action, exercised by the salts of the fluids in which the [225] vibrios are suspended. These vibrios, under the action of media richer or poorer in salts than is the fluid in which they had developed, are said to present an increase of their internal pressure, in consequence of which the vibrios swell up or allow a spherical droplet of protoplasm to escape at one of their poles. This explanation was inadequately supported by its author and cannot be regarded as proved. On the other hand, one is compelled to the conclusion that the granular transformation is due, as we shall see later, to a fermentative action of the peritoneal exudation.

Whilst the vibrios are undergoing this transformation in the

[1] *Ztschr. f. Hyg.*, Leipzig, 1900, Bd. xxxv, S. 1.

peritoneal cavity of an immunised guinea-pig, the animal recovers from a *malaise* that is quite transitory and continues to live, whilst normal unvaccinated guinea-pigs die, an enormous quantity of vibrios swarming in the peritoneal exudation. The difference between the two animals is most striking, and we can readily understand that Pfeiffer was so impressed by it that he was led to attribute the acquired immunity of his guinea-pigs solely to the granular transformation set up by a bactericidal substance contained in the fluids of the immunised animals.

The ease with which we can gain an idea of the change of form in the vibrios under the influence of the fluids of the body, greatly aids the study of the bactericidal substance. Before passing to the question of the part played by this substance in acquired immunity we must consider for a moment the principal properties of this acquired immunity. Very manifest in the peritoneal fluid, the power of causing Pfeiffer's phenomenon is equally evident in the blood serum of immunised guinea-pigs, as has been demonstrated by Bordet. A drop of this serum, when quite fresh, readily and rapidly transforms a number of vibrios into granules. When the serum is kept for several days or has been heated to 55° C. for an hour, the total disappearance of the substance which produces Pfeiffer's phenomenon is brought about. This at once betrays the presence of microcytase in the fluids of guinea-pigs that have acquired immunity against the cholera vibrio. Yet the blood serum and the peritoneal fluid of these animals, having been deprived of their microcytase by heating to 55° or 56° C., still retain a remarkable power over the vibrios. These organisms no longer undergo granular transformation, under
[226] the influence of the heated body fluids, but they are deprived of all power of motion, agglutinate into clumps and acquire a special susceptibility to the action of cytase. Soon after the discovery of Pfeiffer's phenomenon, I[1] was able to demonstrate that this granular transformation can be obtained *in vitro* under the following conditions. Prepare a hanging drop with the blood serum of a guinea-pig vaccinated against the cholera vibrio, a serum which has lost the power of transforming, by itself, the vibrios into granules. Add to it a drop of the peritoneal lymph of a normal unvaccinated guinea-pig; this lymph contains dead or living leucocytes and is, by itself, also incapable of producing Pfeiffer's phenomenon. When, to the mixture of these two fluids, which are inactive when they are employed

[1] *Ann. de l'Inst. Pasteur*, Paris, 1895, t. IX, p. 433.

separately, a few cholera vibrios are added, they are quickly transformed into granules. This transformation, obtained *in vitro*, is remarkably like that produced in the peritoneal cavity of the vaccinated animal.

Jules Bordet[1], working in my laboratory, made a very complete investigation of Pfeiffer's phenomenon outside the animal body and found that, in my experiment, the peritoneal lymph can be replaced by the blood serum of the vaccinated guinea-pig without thereby in the least hindering the granular transformation. After making a specially thorough study of the question he has come to the conclusion that Pfeiffer's phenomenon is the result of the action of two substances. One of these is found in the blood serum and in the peritoneal fluid of guinea-pigs vaccinated against cholera, heated to 55°—56° C. or deprived by some other means of their individual power of transforming vibrios into granules. This substance resists this temperature and only loses its activity on being heated to 68°—70° C. The second of the two substances, that found in the peritoneal lymph or in the blood serum of the normal guinea-pig, is, on the other hand, destroyed at 55°—56° C. and is nothing but the ordinary cytase (or alexine) present in the fluids of normal animals.

The facts we have described with regard to Pfeiffer's phenomenon in the body fluids of immunised animals must, then, be interpreted as follows. The fresh peritoneal exudation or blood serum of these animals readily produces the granular transformation, because in these fluids both the two necessary substances are found. But as microcytase is a very unstable substance which, under the influence [227] of time or heating to 55°—56° C., is destroyed, the fluids of immunised animals are very readily deprived of it. The blood serum then, after being some time outside the body, becomes incapable of transforming vibrios into granules; but when to it is added a small quantity of the cytase, found in the blood serum or in the peritoneal lymph of the normal guinea-pig, the transformation takes place with great rapidity. To the serum of the immunised animal, which has become inactive, is restored its property of setting up Pfeiffer's phenomenon. This interpretation, formulated by Bordet, corresponds to the whole of the known data and is now generally accepted.

As the fluids of immunised animals, that have become incapable of transforming vibrios into granules, still retain their power of rendering these organisms motionless and of uniting them into

[1] *Ann. de l'Inst. Pasteur*, Paris, 1895, t. IX, p. 462.

clumps, it might be asked whether this agglutinative substance might not be the substance, stable under heat, which is necessary for the production of Pfeiffer's phenomenon. For some time, indeed, it was believed that this phenomenon is due to the microcytase acting on vibrios which have first been modified by the agglutinative substance. This latter substance resists heating to 55°—56° C., is only destroyed at higher temperatures, and is retained in the blood serum long after the cytase has entirely disappeared. The analogy between the agglutinative substance of the fluids of animals that have acquired immunity and the substance in the same fluids that is stable under heat is undeniable, and yet these two substances are not identical. A whole series of observations, which we shall presently describe, demonstrate this thesis clearly. A serum may be highly agglutinative without being capable of bringing about the transformation of vibrios into granules; the converse also holds good. The substance which sets up Pfeiffer's phenomenon, and which is found in the fluids of immunised guinea-pigs, is a "fixative substance" analogous to those we have already met with in the serums of animals so adapted that they are able to resorb the various cell elements. As in the resorption of cells, so also in the destruction of micro-organisms, the fixatives are specific. The substance which aids the transformation into granules is not only distinct from the fixatives which sensibilise red blood corpuscles or spermatozoa, but also from the fixatives which sensibilise bacteria. This specificity has been demonstrated and carefully studied by Pfeiffer, who has shown that it may even serve to distinguish species
[228] of bacteria. The serum of a guinea-pig which has been immunised against the cholera vibrio, will render sensitive these vibrios, and these only, to the action of the microcytase. Even allied vibrios, such as various water vibrios, for example, are not sensitive to the fixative of anticholera serum. On the other hand, the serums obtained after the inoculation of these aquatic vibrios are incapable of producing granular transformation in the cholera vibrio.

When we inject into one and the same animal several species of vibrios we obtain a serum or a peritoneal fluid which produces Pfeiffer's phenomenon with the vibrios of all the species which have been used to make the inoculations. This antivibrio serum contains only a single cytase for the vibrios, but contains as many different fixatives as there were species inoculated.

The transformation of vibrios into granules, when produced in a high degree against virulent vibrios, under the influence of the body

fluids of immunised animals, affords a very valuable indication of the simultaneous presence of cytase and of specific fixative. As we have already stated, at the commencement of this account of the acquired immunity of guinea-pigs against the cholera vibrio, Pfeiffer's phenomenon is manifested in the peritoneal fluid of these animals in a very short time (5 to 20 minutes) after the inoculation of the vibrios. This proves that in this fluid the two substances really occur together, and that the fixative and the cytase are in solution in the plasma of the exudation. Is it the same in every part of the body of immunised guinea-pigs? If, instead of introducing the cholera vibrio into the peritoneal cavity, we inject it into the subcutaneous tissue or into the anterior chamber of the eye of these animals, Pfeiffer's phenomenon does not make its appearance. The vibrios, isolated or collected into small clumps, do not undergo granular transformation; they keep their normal vibrio form and remain alive much longer than in the peritoneal cavity. Some of them may be found still living 24 hours after subcutaneous injection and several (4—6) days in the anterior chamber of the eye. Nor can Pfeiffer's phenomenon be observed when the cholera vibrio is introduced into the oedema of the foot, produced in consequence of the slowing of the circulation, the vibrios remaining alive for a fairly long time. These facts clearly indicate that in the fluid thrown out in passive oedema, just as in the aqueous humour of the eye or in the subcutaneous tissue, the two substances necessary to set up the granular transformation are not present. Are both of them absent or only one? This question is easily answered [229] on adding to the fluids mentioned normal guinea-pig's serum, a serum which, by itself, is incapable of producing Pfeiffer's phenomenon. Bordet[1] has made these experiments and found that when to the fluid of the passive oedema of the immunised guinea-pig normal serum is added, this fluid transforms the cholera vibrio into granules, but does so in less degree than does the serum of the same immunised guinea-pig, when heated to 55°—56° C., to which normal serum has likewise been added. There is, then, reason to conclude that the fluid of the oedema does not contain any cytase, but contains a certain quantity of cholera fixative, less, however, than that which is found in the blood serum. As to the aqueous humour of the eye of immunised animals, analogous experiments have demonstrated that it contains neither of the two substances necessary for the production of Pfeiffer's phenomenon.

[1] "Contribution à l'étude du sérum chez les animaux vaccinés," *Ann. Soc. d. sc. nat. et méd. de Bruxelles*, 1895, t. IV.

With the help of the facts I have here summarised, we arrive at the following conclusion. In the animal that is immunised against the cholera vibrio, microcytase is found in the peritoneal exudation; it does not pass, however, either into the fluid of the passive oedema or into the aqueous humour of the eye; the cholera fixative is found in the peritoneal fluid and passes into the oedema, but does not penetrate into the fluid of the eye. This indicates that microcytase is found in fluids rich in leucocytes, but is absent from those which contain very few or none of these cells.

The introduction of vibrios into the peritoneal cavity of immunised guinea-pigs at once produces Pfeiffer's phenomenon, and at the same time causes the disappearance of the majority of the leucocytes from the peritoneal lymph. We have already had occasion, several times, to speak of this phagolysis, because it is produced as a sequel to the injection into the peritoneal cavity of blood, spermatic fluid, and all kinds of fluids. The greater the quantity of fluid injected and the greater the difference of the temperature between it and the contents of the normal peritoneum the more vigorous is phagolysis.

Pierallini[1], working in my laboratory, studying phagolysis in the peritoneal cavity of the guinea-pig, has obtained several results worthy of attention. Of all the fluids used by him, such as water, broth, filtered cultures of micro-organisms and physiological saline
[230] solution, the last of these caused the least intense phagolysis, yet one sufficiently well marked. Immediately after the injection of any of the above fluids the number of leucocytes in the peritoneal lymph diminishes very considerably, the cells being found collected in clumps on the omentum. Many of them exhibit signs of enfeeblement and of partial destruction. Alongside the leucocytes are found fibrinous masses, this affording evidence that some of the leucocytes have been greatly damaged and have given up the fibrin-ferment which induces coagulation of the fibrin. When Pierallini injected fluids containing coloured powders in suspension, such as Indian ink and vermilion, he observed that these substances accumulated on the greater omentum, which became stained black or red. Microscopical examination revealed the existence of a not very intense phagocytosis and a number of free coloured granules in the midst of filaments of fibrin.

The leucocytes which, during this phagolysis, allowed the fibrin-ferment to escape might also give up a certain amount of their microcytase. This microcytase would pass into the peritoneal fluid

[1] *Ann. de l'Inst. Pasteur*, Paris, 1897, t. XI, p. 308.

and give rise to Pfeiffer's phenomenon. If this hypothesis be correct, the suppression of phagolysis would result in the absence of the transformation of the vibrios into granules. It is not a difficult matter to verify this hypothesis as we have a means of preventing phagolysis or at least of reducing it very considerably. Issaeff[1], in an investigation carried out in Pfeiffer's laboratory, demonstrated that an intraperitoneal injection of physiological salt solution, broth, urine, etc., reinforces the leucocytes and brings them up in large numbers into the peritoneal cavity. It is easy to foresee that such an injection would serve to diminish the intensity of the phagolysis. In fact, if we first inject a few cubic centimetres of physiological salt solution or of fresh broth into the peritoneal cavity of a guinea-pig, and if, on the following day, we repeat the same operation, we shall find that after the second injection phagolysis is much less powerful than after the first. Pierallini, who repeated these experiments, observed that the phagocytosis of the coloured granules is much more complete in the guinea-pigs that were treated by a preliminary injection into the peritoneal cavity. The amount of fibrin on the omentum is in this case much less, and the phenomena as a whole show that in these guinea-pigs the damage to the leucocytes is very [231] considerably attenuated.

We have been able to demonstrate that in the case where phagolysis is thus diminished, Pfeiffer's phenomenon is not produced or is manifested in a very feeble degree. If the experiment succeeds, the fluid taken from the peritoneal cavity of a guinea-pig prepared the day before and then injected with a culture of cholera, is opaque and thick, like pus. It contains a mass of leucocytes in good condition, a large number of which gorge themselves in a few minutes with a number of vibrios. The plasma of this exudation contains few vibrios, and these retain their normal form and do not exhibit, save exceptionally, a granular change. A little later there remain no free vibrios ; they are all contained within leucocytes. Pfeiffer[2] declared himself against the facts I have just summarised, because he was never able to prevent the granular transformation of the vibrios, in spite of the preparatory injection of sodium chloride. Abel[3], who repeated the experiments, expressed an intermediate view: in guinea-pigs prepared by injections the day before, he observed that one

[1] *Ztschr. f. Hyg.*, Leipzig, 1894, Bd. XVI, S. 287.
[2] *Deutsche med. Wchnschr.*, Leipzig, 1896, S. 120.
[3] *Centralbl. f. Bakteriol. u. Parasitenk.*, I^te^ Abt., Jena, 1896, Bd. XX, S. 761.

portion of the vibrios became transformed into granules, whilst another became the prey of the leucocytes. The fact is, the suppression of phagolysis demands special conditions: the broth that is injected must be freshly prepared, and before its introduction into the peritoneal cavity it must be heated to 37°—39° C. Even when these precautions are taken it sometimes happens that the experiment is not very successful. In making the experiment we must be guided by the appearance of the peritoneal fluid withdrawn into the small glass pipettes. If the fluid which enters the tube is clear or scarcely clouded, it indicates that phagolysis has taken place, in spite of the preparatory injection. The experiment is successful in those cases where the peritoneal exudation is very cloudy and resembles pus.

As the demonstration of the suppression of Pfeiffer's phenomenon as well as that of phagolysis is of fundamental importance, I asked M. Garnier[1] to carry out further experiments with the object of setting the question at rest. Using a whole series of fluids for the preparatory injection he found that fresh broth gives the best
[232] results. In guinea-pigs in which the phagolysis had been reduced to a minimum, phagocytosis commenced immediately after the injection of the vibrios. In from two to five minutes many vibrios are found inside the leucocytes, the free vibrios now being few in number and not exhibiting Pfeiffer's phenomenon. Garnier in his memoir gives photographic reproductions of leucocytes crammed with vibrios; these should convince the veriest sceptic. Since the publication of this paper no objection has been advanced, and this question of the suppression of the granular transformation of vibrios may now be regarded as definitely settled. I have since demonstrated this feature to many observers, all of whom have assured themselves of its accuracy. It must, then, be accepted that Pfeiffer's phenomenon is not produced in the peritoneal cavity except when there is phagolysis. As this fact renders it very probable that the microcytase, which is necessary for the transformation of the vibrios, escapes from the injured leucocytes, it becomes necessary to verify this hypothesis by a series of other experiments. If this hypothesis be well founded, Pfeiffer's phenomenon should not be observed in those situations in the body where there are no, or almost no, leucocytes already present. These conditions can be realised by injecting cholera vibrios into the subcutaneous tissue or into the anterior chamber of the eye of guinea-

[1] *Ann. de l'Inst. Pasteur*, Paris, 1897, t. XI, p. 767.

pigs that are well vaccinated against the cholera vibrio. Under these conditions, as I have demonstrated in my work on the extracellular destruction of cholera vibrios, the vibrios retain their normal form and are never transformed into granules. Pfeiffer has questioned this result, stating that beneath the skin of vaccinated guinea-pigs the granular transformation is always produced, though in a more feeble fashion and after more delay than in the peritoneal cavity. The contradiction between Pfeiffer's experiments and my own can, however, be explained. When inoculating the vibrios into the subcutaneous tissue, or during the withdrawal of the exudation formed at the point of infection, small haemorrhages are sometimes produced and a certain amount of microcytase is set free from the leucocytes found in the effusion of blood; these cells also give up to the extravasated blood a portion of their fibrin-ferment. When the experiment is successful, that is to say when no haemorrhage is produced during the operations involved, the subcutaneous exudation contains normal vibrios only, without the appearance of any trace of Pfeiffer's phenomenon in the fluid.

If the extracellular transformation of the vibrios into granules [233] were the real cause of the acquired immunity, the absence of this phenomenon in the subcutaneous tissue of the vaccinated guinea-pig should lead to the death of the animal. As a matter of fact this does not take place and the animal resists the inoculation of the vibrios. This conclusion is open to one serious objection. As the cholera vibrio in the great majority of cases is incapable of producing a fatal infection when inoculated subcutaneously, even in normal unvaccinated guinea-pigs, this example of immunity must be placed in the category of natural immunity, a kind of immunity which may depend on causes other than those on which acquired immunity depends. To answer this objection it was necessary to select a race of vibrios capable, when injected subcutaneously, of causing death. Mesnil[1], chief of my laboratory staff, undertook to carry out experiments with the Massowah vibrio, which is regarded by some authors as belonging to the true cholera species. When inoculated subcutaneously into unprotected guinea-pigs, it induces local oedema, in which the vibrios swarm; the infection rapidly becomes generalised and causes the death of the animal in 24 hours. Yet this vibrio, when injected into the subcutaneous tissue of well vaccinated guinea-pigs, is completely resisted by these animals and not the least trace of Pfeiffer's phe-

[1] *Ann. de l'Inst. Pasteur*, Paris, 1896, t. x, p. 375.

nomenon is produced. Under these conditions, a certain number of the vibrios are at first united into masses, but a considerable number remain isolated and motile. Some hours after inoculation the number of clumps diminishes and the isolated vibrios become more numerous, a fact which indicates a certain amount of adaptation of the vibrio to the medium in which it now finds itself. But never, so long as the vibrios remain free in the subcutaneous exudation, do they become transformed into granules.

Salimbeni[1], in an investigation carried out in my laboratory, endeavoured to satisfy himself whether or no Pfeiffer's phenomenon is produced in the subcutaneous tissue of a horse that had been hyperimmunised against the cholera vibrio. This animal had, during a period of 14 months, received considerable quantities of these micro-organisms, and the serum of its blood transformed the vibrios into granules with great rapidity and intensity. In spite of such favourable conditions for the manifestation of Pfeiffer's phenomenon, it was never produced beneath the skin of this horse. The vibrios when injected in this position became completely motionless in a
[234] very short time, but they kept their vibrio form and remained alive for a number of hours. The exudation drawn off up to 24 hours after inoculation still gave growths of the cholera vibrio.

As it is more easy to introduce, without effusion of blood, the cholera vibrio into the anterior chamber of the eye than beneath the skin, and as the aqueous humour contains no fixative, the absence of the granular transformation in the first of these two situations has been observed even by Pfeiffer himself. The demonstration of this fact presents no difficulty, and for a considerable period we may observe free and perfectly motile vibrios moving about in the aqueous humour. The exudation from the eye contains many of these living organisms, which when sown on culture media made their appearance as colonies even when the fluid has been withdrawn from the eye several days after inoculation.

These carefully established facts show very clearly that the micro-cytase is only met with in the fluids of the living animal in those situations in which there are many pre-existing leucocytes and under conditions in which the cells undergo a more or less marked phago-lysis. This may be corroborated by a decisive experiment. When we inject a suspension of the cholera vibrio directly into the veins of a guinea-pig, well vaccinated against these organisms, and whose

[1] *Ann. de l'Inst. Pasteur*, Paris, 1898, t. XII, p. 199.

serum produces *in vitro* Pfeiffer's phenomenon with great rapidity, this phenomenon is not manifested. This experiment has been performed and described by Bordet[1]. Having injected a suspension of this vibrio into the jugular vein of a guinea-pig vaccinated against the cholera vibrio, he killed the animal an hour later and found, in the blood of the heart, vibrios that had kept intact their form and their property of staining with methylene blue. Cultivation of the blood of the heart, liver and spleen gave growths of vibrios. In another guinea-pig, hypervaccinated against the same organism and inoculated by the same method, the blood drawn off shortly (4—15 minutes) afterwards showed, in preparations treated with methylene blue, well-stained vibrios, of normal form and quite intact. This is the most direct proof of the absence of Pfeiffer's phenomenon in the blood fluid of a living animal that enjoys a very [235] marked acquired immunity. The intact vibrios were lodged inside the leucocytes.

Levaditi[2] repeated these experiments in my laboratory and varied the conditions under which the vibrios were injected into the blood vessels. He was sometimes able to observe phagolysis of the leucocytes of the blood and their almost complete disappearance from the the peripheral circulation. In these cases the injured leucocytes accumulated in the pulmonary capillaries and masses of them were seen surrounding groups of vibrios that were transformed into granules. It was, however, easy to exclude phagolysis by preparing the animals by means of injections of physiological saline solution or broth. Under these conditions the leucocytes remained in the blood current and very soon ingested the vibrios. Whilst the vibrios that were still free in the blood plasma retained their form and staining power intact, those found inside microphages were already, in great part, transformed into granules. The rapidity with which these phagocytes ingest the vibrios and set up the changes in them is really extraordinary.

In this case, which affords a typical example of the reaction of the animal organism in acquired immunity, we see a very marked and immediate phagocytosis. It is this same process that has already been described as occurring in the peritoneal cavity of vaccinated guinea-pigs in which phagolysis was absent as the result of preparatory injection. In the subcutaneous tissue and in the anterior

[1] *Ann. Soc. d. sc. méd. et nat. de Bruxelles*, 1895, t. IV.
[2] *Ann. de l'Inst. Pasteur*, Paris, 1901, t. XV, p. 894.

chamber of the eye, where Pfeiffer's phenomenon is regularly absent, the phagocytosis follows its ordinary course and causes the destruction of the vibrios. This result has been confirmed repeatedly—see works by Bordet, Mesnil and Salimbeni already quoted.

We need only compare the extension of Pfeiffer's phenomenon and that of phagocytosis in animals that are immunised against the cholera vibrio, to satisfy ourselves that the former phenomenon is a limited one whilst the latter is general. There might be advanced against the latter conclusion the fact of the absence of any ingestion of the vibrios in the peritoneal fluid of guinea-pigs that are im-
[236] munised but are not preserved against phagolysis. When a little of the peritoneal fluid is drawn off with small tubes shortly after the injection of vibrios into the peritoneal cavity, as a matter of fact, only a very intense Pfeiffer's phenomenon is seen, phagocytosis being completely or almost entirely absent. But this procedure is insufficient. If we are to get an idea of what really takes place in the abdominal cavity, the animal must be killed and the peritoneum and especially the omentum very carefully examined. As first demonstrated by Max Gruber[1] and later by Cantacuzène[2], the greater omentum is, in these cases, covered with a thick layer which contains a large number of leucocytes, of which some are filled with vibrios; further, this layer contains a mass of vibrios, in part transformed into granules, in part agglutinated or isolated and retaining their vibrionic form intact. As time goes on, the phagocytosis becomes more and more marked, and it is impossible to deny its existence or to attribute to it merely a secondary part.

We have seen that the suppression of Pfeiffer's phenomenon in the peritoneal cavity and in the blood, or its total absence in the anterior chamber of the eye, does not in the least deprive the vaccinated guinea-pig of its acquired immunity. The animal resists the vibrios perfectly, without these requiring to be transformed into granules in the body fluids. This transformation does take place undoubtedly, but only inside the phagocytes. As already stated in the discussion on natural immunity (Chaps. VI, VII) the vibrios, after being ingested by the microphages, almost immediately undergo within these cells a change in form, very similar to that observed in the real Pfeiffer's phenomenon. The microphages are often full of a quantity of granules, derived from the ingested vibrios, which in

[1] *München. med. Wchnschr.*, 1896, SS. 277 and 310.
[2] *Ann. de l'Inst. Pasteur*, Paris, 1898, t. XII, p. 273.

a short time are completely digested. This fact, of such constant occurrence in the phagocytosis of the vibrios, furnishes us with still another proof of the microphagic origin of microcytase.

If Pfeiffer's phenomenon is merely a particular instance of the transformation of vibrios into granules in fluids containing microcytase, it is quite natural that its suppression should not involve a fatal infection of the vaccinated animals. On the other hand, if the phagocytic reaction, so widely different, really plays an important part in acquired immunity, everything that interferes with phagocytosis must at the same time compromise the refractory condition. With [237] the object of solving this question Cantacuzène[1], working in my laboratory, undertook a detailed investigation of this point. He showed that the injection of opium, in a non-fatal dose, narcotised the guinea-pig and at the same time prevented the movements of the leucocytes. Small glass tubes containing cholera vibrios and introduced beneath the skin of vaccinated guinea-pigs, became filled with numbers of leucocytes in the non-narcotised animal; in the guinea-pig that had received tincture of opium, the tubes left for several hours contained no leucocytes and later only did they begin to enter the tubes. When a strong dose of cholera vibrios was injected into the peritoneal cavity of thoroughly vaccinated guinea-pigs, the animals easily resisted the inoculation. When, however, similarly vaccinated guinea-pigs were submitted to the influence of tincture of opium, the same dose of vibrios caused their death. In these narcotised animals, in spite of the considerable dilatation and hyperaemia of the vessels and in spite of the marked hyperleucocytosis of the blood, diapedesis was not produced during the first few hours after the injection of theopium, and it was not till later (5 to 6 hours after injection) that the leucocytes began to appear in the peritoneal cavity. The vibrios profit by the period of inactivity of the phagocytes to multiply, retaining their motility and also the property of staining with basic aniline dyes. When the retarded leucocytes make their appearance in the peritoneal cavity, they find it already invaded by a multitude of vibrios. In spite of this the leucocytes, especially the microphages, ingest an enormous number of the organisms; this does not prevent the death of the guinea-pigs, though it takes place some hours later than in the unvaccinated control animals. At the moment of death, free vibrios are no longer found in the exudation; they have all been ingested by the microphages, inside

[1] *Ann. de l'Inst. Pasteur*, Paris, 1898, t. XII, p. 288.

which they have undergone granular transformation. On making a post-mortem examination of the animal a large number of small heaps of vibrios, such as are never met with in animals that have not been submitted to the action of opium, are found on the omentum.

All that is necessary, then, is to retard the phagocytic reaction for a few hours in order to cause well-vaccinated guinea-pigs to succumb to the action of the vibrios. One can readily understand
[238] that, with this result before us, there can be no hesitation in attributing to phagocytosis a much more important part in assuring acquired immunity than to Pfeiffer's phenomenon.

The study of other diseases produced by vibrios only serves to corroborate the general conclusions that follow from the detailed study of the essential processes in acquired immunity against the cholera vibrio. It is here necessary to recall the discovery by v. Behring and Nissen of the very marked bactericidal power of the blood serum of guinea-pigs that have been vaccinated against Gamaleia's vibrio. When this fact was first demonstrated we were justified in thinking that the vibrionicidal property of the blood might by itself explain this acquired immunity; but a comparative study of the phenomena which take place *in vitro* with those which take place in the living animal, soon demonstrated how slight was the foundation for this hypothesis. Whilst the vibrios, when sown in the blood serum of hypervaccinated guinea-pigs, there perished in large quantities or even the whole of them, these same organisms, when inoculated into the subcutaneous tissue of the same animals, remained alive for several days. Gamaleia's vibrio is much less capable of being transformed into granules than is the cholera vibrio, and we find it retaining its normal form even inside the leucocytes. There is no occasion in this case, therefore, to look for Pfeiffer's phenomenon.

The rapid and marked destruction of Gamaleia's vibrio, *in vitro*, in the blood serum of vaccinated guinea-pigs, and the prolonged survival of these organisms in the living animal, afford additional evidence that the two groups of phenomena cannot be identical. On the other hand, it furnishes a further proof that, during the preparation of the serum, there is produced, parallel with the coagulation, another process which confers bactericidal power on the serum. It is quite evident that, as in the case of the cholera vibrio, we have here to do with the liberation of microcytase at the expense of the destroyed or injured leucocytes. Acting along with the specific fixative of the body fluids, this cytase causes the death

of the vibrios introduced into the serum. In the living organism, the microcytase not being free, these vibrios, although influenced by the fixative, resist until they have become the prey of the phagocytes. In an investigation which was the subject of a communication to the International Congress of Hygiene in London in 1891[1], I demonstrated that the phagocytic reaction is produced with [239] great intensity in guinea-pigs that have been vaccinated against Gamaleia's vibrio. The inoculation of this organism into the subcutaneous tissue, an inoculation which sets up a rapidly fatal infection in untreated guinea-pigs, gives rise in immunised animals to the formation of an abundant exudation, in which the numerous vibrios soon meet with resistance from the phagocytes. These phagocytes ingest the living vibrios, retaining them for some considerable time in their interior, but in the long run always digesting them completely. During the last phase of this struggle, we sometimes find, inside the leucocytes, vibrios that are transformed into spherical granules. It was with these cells, filled with ingested vibrios, that I was able first to carry out an experiment that has since been repeated again and again, always with the same result. When from a well-vaccinated guinea-pig a drop of subcutaneous exudation is withdrawn, at a stage when all the vibrios have for some time been ingested by the leucocytes, and transferred, in the form of a hanging drop, to the incubator at 35°—37° C., it is found that the ingested vibrios develop inside the phagocytes which have died outside the animal. The vibrios first fill the leucocyte and, continuing to multiply, cause the cell to burst when they distribute themselves in the fluid of the hanging drop (figs. 40 and 41). This experiment proves, in the first place, that the vibrios have been ingested alive, and, secondly, that the plasma of the exudation was incapable of preventing their later development.

Having summarised the principal phenomena exhibited by vibrios in an animal possessing acquired immunity, we must now enquire whether the mode of destruction and disappearance taking place in these vibrios is of general application. Naturally, we commence this study with the spirilla, which in many respects present a great analogy to the vibrios. The task is an easy one, thanks to a very careful work recently published by Sawtchenko[2], on the *Spirochaete*

[1] [*Trans. Seventh Internat. Congr. of Hyg. and Demogr.* London, 1892, Vol. II. p. 179;] *Ann. de l'Inst. Pasteur*, Paris, 1891, t. v, p. 465.

[2] *Arch. russes de Pathol.*, etc., St Pétersb., 1900, t. IX, p. 584; Sawtchenko et Melkich, *Ann. de l'Inst. Pasteur*, Paris, 1901, t. XV, p. 503.

obermeyeri of recurrent fever. We know, from what has been said in Chapter VI, that the spirochaetes found in the serum of persons

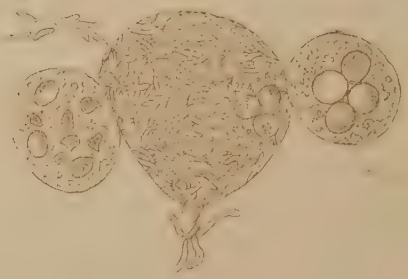

Fig. 40. Vibrios (*V. metchnikovi*) developed inside a microphage from a vaccinated guinea-pig.

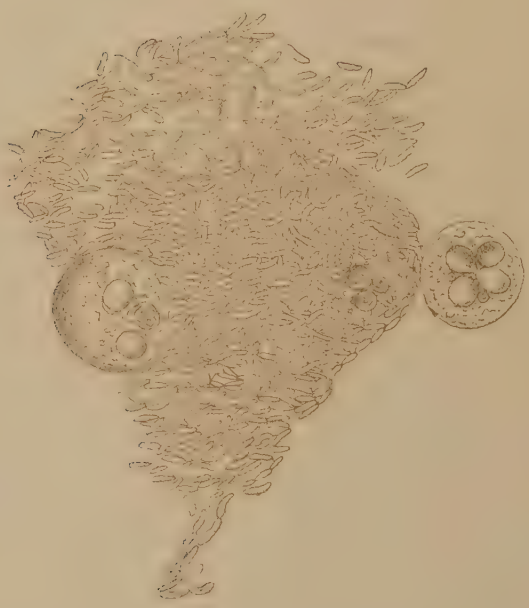

Fig. 41. Vibrios (*V. metchnikovi*) developed in a drop of exudation from a vaccinated guinea-pig. The vibrios have ruptured the microphage and scattered themselves in the fluid.

attacked by this organism, are, in the peritoneal cavity of guinea-pigs, destroyed by the intervention of the macrophages. These
[240] phagocytes guarantee the natural immunity of the guinea-pig against

the parasite of recurrent fever. In guinea-pigs, into which blood or serum containing spirilla has been injected on several occasions, the destruction of these micro-organisms is effected in a different [241] way. When Sawtchenko introduced a number of *Spirochaete obermeyeri* into the peritoneal cavity of guinea-pigs so prepared, he noted that they underwent a transformation resembling that observed in Pfeiffer's phenomenon. In a short time the majority of these micro-organisms assumed the form of very delicate spirilla to which were attached round granules. There was not a complete transformation of the spirilla into granules, but a portion of their contents exuded in the form of spherical drops. The spirilla that exhibited these changes lost their motility and collected into clumps. There was undoubtedly an extracellular transformation of the spirilla, but this took place only in the peritoneal cavity. When injected into the subcutaneous tissue of a prepared guinea-pig the spirilla brought about the formation of a firm but scanty exudation in this situation. In this exudation were found leucocytes containing spirochaetes which retained their normal form. These micro-organisms were found exclusively in macrophages and gave no evidence of the occurrence of Pfeiffer's phenomenon. A like absence of this phenomenon was observed in normal guinea-pigs which had been injected subcutaneously with the same quantity of spirilla. In these animals, however, the oedema that appeared at the seat of inoculation was abundant and soft, and the disappearance of the spirilla, that is to say their ingestion by the macrophages, took place at a very much later period than in the prepared guinea-pigs. We have, therefore, in this respect a complete analogy with the vibrios: in both cases there is an absence of granular transformation below the skin and an ingestion by the leucocytes of the exudation; on the other hand, we have Pfeiffer's phenomenon appearing in the peritoneal fluid. This analogy extends even further. Thus, in guinea-pigs prepared by repeated injections of human serum rich in spirilla, Sawtchenko could suppress Pfeiffer's phenomenon in the peritoneal cavity just as easily as in the case of the vibrios. All he had to do was to inject a certain quantity of broth into the peritoneal cavity of his immunised guinea-pigs. Twenty-four hours later, on introducing spirilla into the animals at the same site, they retained their motility for hours, did not exhibit any granular transformation and were ultimately completely ingested by the macrophages.

These facts lead us to conclude that the fate of the spirochaetes

[242] of recurrent fever in the organism of guinea-pigs prepared by previous injections is governed by laws the same as those established for acquired immunity against vibrios. The spirilla are ingested and destroyed by the phagocytes, except where phagolysis occurs, in which case the cytase, being set free, attacks the micro-organisms outside the leucocytes.

After his discovery of the granular transformation of vibrios, R. Pfeiffer, in collaboration with several of his pupils, set himself to discover how far this phenomenon was general in acquired immunity. He directed his attention to the typhoid cocco-bacillus, upon which he had already published[1] a very detailed account of work carried out in conjunction with Kolle. These observers availed themselves of the discovery made by Beumer and Peiper[2], and Chantemesse and Widal[3] and confirmed by other observers, that laboratory animals, especially mice and guinea-pigs, could be easily vaccinated against the fatal disease set up by the micro-organism of typhoid fever. As in the experimental infection of the guinea-pig by the cholera vibrio, the vaccination of the animals against the typhoid bacillus could be carried out very easily, either by using sterilised cultures or the fluids of cultures deprived of their organisms by filtration. In the small laboratory animals a most marked acquired immunity may thus be obtained, and the study of the phenomena which appear in the vaccinated organism afforded evidence of a general analogy with those which have been observed when vibrios are used. In the peritoneal cavity of the immunised guinea-pigs, Pfeiffer's phenomenon proper does not appear, that is to say, only a few of the bacilli are transformed into granules, the large majority retaining their bacillary form; still they are evidently greatly damaged: they become motionless and agglutinate more or less completely into clumps. If, however, a few of these micro-organisms are sown on nutritive media, they multiply freely and give abundant growths. The peritoneal fluid, then, acts most unmistakably upon the typhoid bacillus, but in a much less degree than does the peritoneal exudation of guinea-pigs upon the cholera vibrio when immunised against that organism.

[243] In both cases we have a pronounced phagolysis which sets free the microcytase, whose action on the vibrio is more marked than on the bacillus of typhoid fever. This extracellular action on the

[1] *Ztschr. f. Hyg.*, Leipzig, 1896, Bd. XXI, S. 203.
[2] *Ibid.* 1887, Bd. II, S. 110.
[3] *Ann. de l'Inst. Pasteur*, Paris, 1892, t. VI, p. 755.

typhoid bacillus in the peritoneal cavity can be easily prevented by a previous injection, twenty-four hours before, of broth, physiological salt solution, or normal serum. The suppression of phagolysis is, as in the case of vibrios and spirilla, followed by the suppression of extracellular action on the typhoid bacilli.

The same analogy is observed in the phenomena which appear beneath the skin. The bacillus of typhoid fever, when introduced into the subcutaneous tissue of vaccinated guinea-pigs, although not appreciably injured by the fluid of the exudation, undergoes some agglutination. The injurious action of the fluids of the body is here still less effective than in the peritoneal cavity. But, as in the peritoneal cavity of vaccinated guinea-pigs previously treated with broth, so in the subcutaneous exudation it is the phagocytes which destroy the micro-organisms. In both cases there is a very great afflux of leucocytes, mainly microphages. These cells ingest and digest the bacilli, which ultimately disappear. The micro-organisms ingested by the microphages, once inside these phagocytes are transformed into granules very like those observed in the cholera vibrio similarly treated. In this respect the analogy between the two micro-organisms is complete.

Oppel, working in my laboratory, has repeated Cantacuzène's work on the retarding action of opium upon the phagocytic process. He obtained the same results: under the influence of the narcotic, the leucocytes intervened only at a late stage, with the result that the vaccinated guinea-pigs succumbed to the typhoid infection. The same conclusion must be drawn from the experiments made by A. Wassermann[1]. Guinea-pigs that had been immunised against the bacillus of typhoid fever were completely resistant to a dose that was always fatal to the control animals. When, however, along with this dose of bacilli, a certain quantity (3 c.c.) of a serum which hinders the phagocytic reaction is injected, the guinea-pigs lose their immunity and die from typhoid infection. The serum employed by Wassermann was obtained from rabbits that had been treated with the blood serum of guinea-pigs. Rabbit's serum, thus prepared, neutralises the action of the guinea-pig's cytase, but, as demonstrated [244]
by Besredka[2], it also exercises several other functions, one especially, that of preventing phagocytosis. In Wassermann's experiments it was the antiphagocytic function, then, that was the important factor

[1] *Ztschr. f. Hyg.*, Leipzig, 1901, Bd. xxxvii, S. 173.
[2] *Ann. de l'Inst. Pasteur*, Paris, 1901. t. xv, p. 209.

in the suppression of the acquired immunity of the guinea-pigs. These experiments supply a fresh proof of the great importance of the phagocytic reaction in this kind of immunity, and afford further confirmation of the analogy between the mechanism of resistance of the animal's organism against the typhoid bacillus and that against the cholera vibrio.

In presence of this striking analogy, it is unnecessary to insist further on the details of the acquired immunity of animals against the experimental disease set up by the micro-organism of typhoid fever. It will be better to select another example from the group of bacilli. Let us first take the acquired immunity against the bacillus of blue pus (*Bacillus pyocyaneus*) which for many years has been regarded as the best example in which to study this kind of immunity. Charrin, who was the first to obtain disease with this bacillus experimentally, published several notes[1] on the acquired immunity of the rabbit against it. He demonstrated the possibility of vaccinating this animal not only with living bacilli, but also with the products of their culture; he studied the blood serum of vaccinated animals, comparing it with the serum of normal rabbits, especially as to its action on the development of the *Bacillus pyocyaneus*. Although unable to find any bactericidal power properly so called in the serum of immunised rabbits, Charrin was the first to draw attention to certain modifications undergone by the bacilli when grown in this medium. He noted that under these conditions no pyocyanin was produced, and, in collaboration with Roger, he demonstrated that, in the serum of the vaccinated rabbit, the *Bacillus pyocyaneus* forms packets composed of little chains of greater or less length, whilst in the serum of the normal, susceptible rabbit, it develops in the form of normal rods, the rods for the most part being isolated.

From his experiments *in vitro* Charrin concluded that there was marked enfeeblement of the functions of the *Bacillus pyocyaneus* when submitted to the action of the vaccinated animal organism.
[245] Bouchard[2] has gone so far as to develop a theory of acquired immunity, in which the principal part is attributed to the impossibility of the micro-organism, after it has invaded the refractory animal, secreting its fluid products; there is no vascular dilatation and diapedesis does not take place. A comparative observation of the

[1] *Compt. rend. Soc. de biol.*, Paris, 1889, pp. 250, 330, 627; 1890, pp. 203, 332, 195.
[2] "Les microbes pathogènes," Paris, 1892.

phenomena observed in rabbits that are susceptible to the pyocyanic disease and of those met with in vaccinated rabbits, most clearly, however, demonstrates the impossibility of accepting Bouchard's interpretation. The inoculation of the bacillus of blue pus below the skin of the ear of the normal (unvaccinated) rabbit sets up extensive inflammatory reaction with marked hyperaemia; the diapedesis of the white corpuscles takes place at a comparatively late stage of the process and phagocytosis is neither set up nor completed until very late. On the other hand, in vaccinated rabbits, infected in the same way, the hyperaemia of the ear is insignificant, but diapedesis occurs very early and phagocytosis commences at once. It is not, therefore, the impossibility for the leucocytes to traverse the vessel wall, owing to the absence of the dilatation of the veins, which prevents them from making their way rapidly to the field of battle; it is their imperfect positive sensitiveness that is accountable for the tardy and incomplete phagocytosis. This interpretation is confirmed in other cases of acquired immunity.

More recently, Paul Müller[1] has laid special stress on the part played by the bactericidal action of the serum of animals that have been vaccinated against the pyocyanic disease. For him the negative results obtained by his predecessors lose their significance, since all their experiments were carried out under conditions of aërobiosis, whilst it is only in the absence of free oxygen that this bactericidal power can be exerted at all freely. Müller, therefore, set himself to compare under anaërobic conditions the bactericidal action on the *Bacillus pyocyaneus* of serums coming from normal and from vaccinated animals. He succeeded in demonstrating that the blood serum of vaccinated animals is more bactericidal than that of normal rabbits. Before, however, drawing any conclusion from this observation, the following question must be answered: Are the phenomena observed *in vitro* comparable with those seen in the living animal? In preceding chapters it has been shown so often that the blood serum obtained after the separation of the extravascular clot, can in no way be identified with the plasma of the circulating blood, [246] that it is unnecessary to argue this matter further. If we wish to gain a clear idea of the mechanism of immunity in the living animal we must observe the course of events in the vaccinated animal and not draw conclusions from observations *in vitro* except after strict examination. All the works on pyocyanic immunity

[1] *Centralbl. f. Bakteriol. u. Parasitenk.*, I^te^ Abt., Jena, 1900, Bd. XXVIII, S. 577.

above summarised lie under the reproach that in them this rule has not been adhered to.

Since the discovery of Pfeiffer's phenomenon in animals that have been vaccinated against the cholera vibrio, much greater care has been taken to attend to the changes that occur in the animal that enjoys acquired immunity. Wassermann[1] was the first to attempt to apply Pfeiffer's discovery to the *Bacillus pyocyaneus*. With a race of this bacillus rendered more virulent he succeeded in producing a fatal experimental malady in the guinea-pig against which he was able by various methods to vaccinate these animals.

He thus describes the phenomena observed in the peritoneal cavity of immunised guinea-pigs. Soon after injection the bacilli of blue pus become motionless, then "the rods swell up and melt, like wax in hot water. The formation of granules, such as occur in the cholera vibrio, has been observed but rarely. The process recalls rather that which takes place in experimental typhoid fever, as described by R. Pfeiffer. In all cases the phenomenon of solution takes place entirely in the fluid of the exudation, without any co-operation on the part of the leucocytes" (p. 284). We see that we have still to do with a kind of attenuated Pfeiffer's phenomenon, without any granular change, but with an immobilisation of the bacilli. As Wassermann has remained satisfied with the examination of the peritoneal content which, as we know, gives but an imperfect picture of acquired immunity, Gheorghiewsky[2] set himself to study the question more thoroughly under my direction. With this object he vaccinated a series of guinea-pigs with living bacilli of blue pus, a sure method of obtaining acquired immunity. On examining the peritoneal fluid (withdrawn shortly after the injection of the bacilli) of the vaccinated guinea-pigs, he found that the bacilli were motionless and had undergone a certain degree of agglutination. They were
[247] not transformed into granules but became thicker and somewhat more dumpy. These changes are observed during the period of phagolysis, when only a few scattered leucocytes are to be found in the fluid of the peritoneal cavity. About two hours after the injection of the bacilli the leucocytes begin to reappear in the peritoneal exudation, more especially the microphages, which lose no time in seizing the bacilli, some of which become transformed into granules. A few hours later the exudation, containing a multitude of leucocytes,

[1] *Ztschr. f. Hyg.*, Leipzig, 1896, Bd. XXII, S. 263.
[2] *Ann. de l'Inst. Pasteur*, Paris, 1899, t. XIII, p. 298.

no longer contains any free bacilli: all are found inside the microphages. Nevertheless, if a drop of the exudation now be withdrawn and kept for some time at a temperature of 37° C., it will be found that the bacilli multiply inside the dead phagocytes outside the animal. We thus obtain colonies of bacilli, a fact which clearly proves that these bacilli whilst still alive have been ingested by the leucocytes. This experiment is, therefore, very similar to the one we have described in connection with Gamaleia's vibrio.

Even at a later period, 24 or 30 hours after the injection of the bacilli, that is to say at a period when an examination of the exudation no longer reveals the presence of bacilli, the sowing of a drop of this exudation on a nutrient medium still gives isolated colonies of the *Bacillus pyocyaneus* capable of producing the characteristic pigments. At a still later period, when the peritoneal exudation remains sterile, a post-mortem examination of the animals enables one to recognise, beneath the peritoneal surface, small white points made up of leucocytes. The sowing of these masses almost invariably gives colonies of the *Bacillus pyocyaneus* which form blue pigments. We see from this account that, even in the peritoneal cavity of vaccinated animals, matters by no means go on in a uniform fashion, as would appear from Wassermann's statements. Some bactericidal action in the peritoneal fluid there certainly is, but it is quite transient, and is limited to the period of phagolysis. The majority of the bacilli resist this attack of the body fluids to continue their struggle with the phagocytes, which, however, ultimately get the upper hand. In the subcutaneous tissue the part played by this phagocytic reaction is still more general. Gheorghiewsky has studied it not only in vaccinated guinea-pigs but also in a goat which had received several large injections of the *Bacillus pyocyaneus*. He observed that shortly after the subcutaneous injection of these bacilli, the fluid which accumulates at the seat of inoculation renders them [248] motionless and in part agglutinates them. This fluid is clear and contains a few leucocytes and a number of bacilli which still retain their usual form. Some time later the leucocytes begin to come up to the seat of inoculation and to ingest the bacilli. At the end of 10 to 15 hours all the bacteria have been seized by the microphages and we no longer find any of them free. A hanging drop of this exudation, transported to the incubator, soon swarms with bacilli which have sprung from the organisms ingested by the leucocytes.

The exudation becomes more and more abundant at the seat of inoculation and ends in the formation of an abscess, from the contents of which cultures of the *Bacillus pyocyaneus* may be obtained for a fortnight. The bacilli, however, finally disappear, this being due to the destructive action of the phagocytes and not to that of the fluid of the exudation.

This fundamental part played by phagocytosis in acquired immunity against the *Bacillus pyocyaneus* has been confirmed by Gheorghiewsky by experiments on guinea-pigs vaccinated and then submitted to the action of opium. As in the analogous experiments of Cantacuzène on the cholera vibrio, the opium narcosis retards diapedesis and this, for some time, increases the chances of the bacilli. A tardy diapedesis and phagocytosis, no doubt, is produced which ends in the ingestion of the bacilli, but the animal loses its acquired immunity and finally succumbs in spite of the fact that the dose of *Bacillus pyocyaneus* was insufficient to kill a control guinea-pig vaccinated to the same degree, but not submitted to the action of opium.

The example we have just analysed relates, then, to a micro-organism which is more resistant than are the vibrios, Obermeyer's spirilla or even the typhoid bacillus, to the action of the microcytase which has escaped from the cells during phagolysis. The *Bacillus pyocyaneus* undergoes, in the fluids of the vaccinated animal, the action of the specific fixative and can thus be rendered motionless and become agglutinated. But this action is insufficient to ensure immunity and should phagocytosis not take place in time to ingest the bacilli, the vaccinated animal succumbs. The reaction of the phagocytes is, therefore, indispensable if the acquired immunity is to be effective. In this respect the analogy is very great between the resistance of the vaccinated animal against the various bacteria (vibrios, spirochaetes, typhoid cocco-bacilli, bacilli of blue pus) that we have so far studied in this chapter. These bacteria have, however,
[249] this in common;—they are all endowed with a considerable power of motion. Pursuing our examination of the principal data on acquired immunity against micro-organisms, we must now choose examples from the group of non-motile bacilli; amongst these we assign the first place to the micro-organism of swine erysipelas. This small bacillus has been the subject of several important researches on acquired immunity, one of which at a certain period caused quite a sensation in the bacteriological world. Emmerich[1], in an investi-

[1] *Fortschr. d. Med.*, Berlin, 1888, Bd. VI, S. 729.

gation carried out in collaboration with di Mattei, made an unexpected announcement. He said he believed that he was justified in affirming that the acquired immunity of rabbits against the bacillus of swine erysipelas is due to the formation, in the fluids of the body, of an antiseptic substance which very quickly destroys this organism. This substance, secreted by the cells of the vaccinated animal, was supposed to act after the fashion of a solution of bichloride of mercury and to kill a large number of bacilli, introduced subcutaneously, in from 15 to 25 minutes. This discovery was not confirmed. In a series of experiments that I carried out[1] with the object of clearing up this question, and made under conditions as favourable as possible for the demonstration of the supposed bactericidal secretion, this action was never manifested. Not only did the virulent bacilli of swine erysipelas, when injected subcutaneously into well vaccinated rabbits, remain alive in the subcutaneous exudation for hours and even days, but the attenuated bacilli of Pasteur's vaccines likewise remained intact. These bacilli when introduced into the anterior chamber of the eye survived for even a longer period. Here, as beneath the skin, the injection of the bacilli induced an exudation rich in leucocytes, amongst which microphages predominated. These phagocytes at once began to seize the bacilli which were destroyed not in the fluid of the exudation but within the leucocytes. Long after all the bacilli had been ingested, 24 hours and more after inoculation, the sowing of the exudation frequently gave growths in appropriate media.

Emmerich[2] sought by new experiments to remove these objections, but he found that the bacilli of swine erysipelas did not disappear [250] from the vaccinated animal until some 8 or 10 hours after they had been introduced. There is, therefore, no longer any question of a rapid bactericidal action at all comparable to that of corrosive sublimate, which would destroy the introduced bacilli in less than an hour. The limit of 8 to 10 hours, accepted by Emmerich, is still too short and is not in accordance with my experiments; but even this was quite sufficient for the appearance of a free phagocytosis, a condition that really occurs. Emmerich has not directed his researches in this direction, and his theoretical conclusions did not in the least weaken the value of my arguments drawn from the demonstration of the ingestion and intracellular destruction of the bacilli by phagocytes.

[1] *Ann. de l'Inst. Pasteur*, Paris, 1889, t. III, p. 289.

[2] *Arch. f. Hyg.*, München u. Leipzig, 1891, Bd. XII, S. 275.

The researches on immunity against swine erysipelas then languished for some time, until the discovery of Pfeiffer's phenomenon gave a fresh stimulus to the study of this problem. One of Pfeiffer's pupils, Voges[1], sought to apply the results obtained in the case of the cholera vibrio to the acquired immunity against the bacillus of swine erysipelas. He studied the blood serum of animals vaccinated against this bacillus and believed himself justified in affirming the existence of an acquired bactericidal power. Under no condition, however, did he observe anything comparable to Pfeiffer's phenomenon, and he was compelled to admit that the bactericidal action of the serum is very feeble and only takes effect on young bacilli whose membranes are as yet very delicate and not very resistant. Mesnil[2] repeated these researches in my laboratory, but his results were very different from those obtained by Voges. The blood serum of rabbits, fully vaccinated against the bacillus of swine erysipelas, proved to be a good culture medium for this bacillus, and Mesnil affirms, as the result of numerous well-established observations, that "*in vitro*, the serum of rabbits immunised against the erysipelas has no bactericidal power or a very insignificant one." On the other hand, the same fluid had a very marked agglutinative power. The bacillus of swine erysipelas, being non-motile, does not present the abrupt change that is observed in vibrios or in the typhoid bacillus when submitted to the influence of specific serums—under which conditions these organisms at once lose their motility. But the bacilli of swine erysipelas, when introduced into the specific serum of vaccinated animals, run together into masses which become
[251] more and more voluminous and fall to the bottom of the vessel, leaving a limpid supernatant fluid. When this bacillus is sown in the serum of vaccinated animals, it is seen to develop in the form of chains, composed of a large number of segments, which fall to the bottom of the tube. These bacilli, however, whether agglutinated or developed in chains, never show any attenuation in virulence. When the serum which bathes them is got rid of by washing, they are just as virulent as are the bacilli developed in the serum of normal unvaccinated rabbits. It is important to show that this virulence is kept up in spite of the fact that the bacilli, when placed in contact with the serum of immunised animals, become permeated

[1] *Ztschr. f. Hyg.*, Leipzig, 1896, Bd. XXII, S. 515; *Deutsche med. Wchnschr.*, Leipzig, 1898, S. 49; *Ztschr. f. Hyg.*, Leipzig, 1898, Bd. XXVIII, S. 38.

[2] *Ann. de l'Inst. Pasteur*, Paris, 1898, t. XII, p. 481.

with the specific fixative, as shown by the experiments of Bordet and Gengou[1]. These observers, indeed, have demonstrated that the bacilli of swine erysipelas, when kept for 24 hours in the specific serum heated to 55° C., acquire the property of absorbing the cytases contained in the unheated serum of normal animals.

The study of acquired immunity against the bacillus of swine erysipelas teaches us that this immunity is not due to any extracellular destruction comparable with Pfeiffer's phenomenon; and that this immunity causes the production of a specific fixative and of a specific agglutinative substance, whose action on the resistance of the animal, to judge from the complete virulence of the bacilli when agglutinated and impregnated by fixative, is feeble or *nil.* It is the phagocytic reaction which is dominant in the immunised animals and which brings about the intracellular destruction of the bacilli.

The history of the anthrax bacillus, another representative of the group of non-motile bacilli, is particularly interesting, the more so that for some time the researches on acquired immunity have been concentrated almost entirely on the analysis of the facts observed in animals that have been vaccinated with the two Pasteur vaccines. In this way a large number of valuable facts have been collected; of these the more important may be presented to the reader.

In my first work on this subject[2] I called attention to the fact that in the rabbit vaccinated against anthrax, the bacilli, when inoculated subcutaneously, soon become the prey of leucocytes which accumulate at the spot menaced. In the unvaccinated control rabbits, however, the anthrax bacilli remain in a free state in the fluid of the subcutaneous exudation, only a few isolated rods being [252] found inside phagocytes. I have since been able to confirm this fact[3], which must now be regarded as fully established. In the vaccinated rabbits the leucocytes exhibit a very marked positive chemiotaxis against the anthrax bacilli, whilst in normal unvaccinated rabbits the chemiotaxis of the leucocytes in the anthrax of the subcutaneous tissue is distinctly negative. When a small quantity of anthrax culture is inoculated subcutaneously into vaccinated and into unvaccinated rabbits there may be observed, even within a few hours, a very great difference. In the former there is found at the seat of inoculation an infiltration which swarms with leucocytes

[1] *Ann. de l'Inst. Pasteur*, Paris, 1901, t. xv, p. 295.
[2] *Virchow's Archiv*, Berlin, 1884, Bd. xcvii, S. 502.
[3] *Virchow's Archiv*, Berlin, 1888, Bd. cxiv, S. 465.

in the act of devouring bacilli. In the normal, susceptible rabbit, on the other hand, the exudation produced is soft, rich in fluid, and very poor in leucocytes. The vessels in the vicinity are distended with blood, and the fact that the leucocytes do not come up to the seat of inoculation is in no way due to the absence of vascular dilatation which might prevent diapedesis. The vessels are much more dilated than in the vaccinated rabbit, and yet in the latter the emigration is incomparably greater. This essential difference must be attributed to the sensitiveness of the leucocytes, which exhibit a negative chemiotaxis in the normal rabbit but a very marked positive chemiotaxis in the vaccinated rabbit.

It has been shown repeatedly that the subcutaneous exudation, very rich in leucocytes which have had time to ingest all the bacilli, when inoculated into guinea-pigs, ensures the appearance in them of a generalised and fatal anthrax; this affords evidence that the phagocytosis is exercised against virulent and therefore living bacilli. Marchoux[1], in Roux's laboratory, has carried out numerous experiments on the vaccination of rabbits and has observed that the inoculated anthrax bacilli cause an exudation very rich in leucocytes, and that these cells ingest and destroy the bacilli. The phagocytes easily rid the refractory animal of the bacilli in the vegetative state, but the spores are much more resistant. After being devoured by the leucocytes they may remain inside them for months without germinating. Marchoux obtained cultures of anthrax from the subcutaneous exudation taken from vaccinated rabbits 70 days after inoculation.

The fact that the bactericidal action of the blood serum on
[253] anthrax bacilli is specially well marked in the rat, suggested the idea of trying to obtain, in this rodent, an augmentation of this property as a result of vaccination. Sawtchenko[2] attempted to do this in an investigation already cited in Chapter VI, carried out in my laboratory. He succeeded in thoroughly vaccinating white rats against virulent anthrax and in showing that the blood serum of these animals rendered refractory "is bactericidal in the same degree as that of non-immunised rats." In the vaccinated rats "the subcutaneous exudation was as free from bactericidal substances as was the lymph of the control animals." Sawtchenko was unable to demonstrate any increase of bactericidal power except in the peritoneal exudation of rats vaccinated by injection of cultures into the peritoneal cavity.

[1] *Ann. de l'Inst. Pasteur*, Paris, 1895, t. IX, p. 805.
[2] *Ann. de l'Inst. Pasteur*, Paris, 1897, t. XI, p. 881.

In spite, however, of the absence of any increase in the bactericidal property of the blood serum and of the subcutaneous exudation in vaccinated rats, the cell reaction obtained in them is very different from that met with in normal, susceptible rats. In a very short time (3 to 5 hours) after the subcutaneous injection of anthrax bacilli into the control rats (susceptible), an evident oedema is produced; in the vaccinated rat there is none. The exudation, not very abundant in the latter, already contains a number of leucocytes which are actively phagocytic, whilst in the control animal, examined simultaneously, "leucocytes are rarely met with, and few of them contain bacilli." Later, the difference becomes still more marked. Pronounced oedema occurs in the control animal, it is poor in leucocytes but rich in bacilli, which continue to multiply; but "in the immunised rat, we find not a clear exudation but a thick and purulent fluid, full of leucocytes." These cells devour all the bacilli; not a single one remains free. "Even after 14 hours bacilli ingested by the leucocytes are present and a culture of anthrax bacilli may be obtained from fluid taken from the seat of inoculation. Further, guinea-pigs or rats, when inoculated with a drop of this exudation (which contains no anthrax spores), succumb to anthrax."

Even before these researches on the immunity of rats had been carried out, an attempt had been made to gain some idea of the differences presented by the vaccinated fluids of animals as compared with those presented by the fluids of control animals susceptible to anthrax. In 1886 I was able to demonstrate[1] that the anthrax [254] bacillus develops abundantly in the defibrinated blood of sheep that had acquired immunity as the result of vaccination by Pasteur's method. When these bacilli contain spores and are inoculated into rabbits they rapidly produce a fatal anthrax; but when no spores are present the injection of bacilli does not produce a fatal disease, and such infection is well supported by the rabbits. From this I concluded at that time that the anthrax bacillus must, in the blood of the vaccinated sheep, undergo a real attenuation in virulence, an interpretation which, as will be seen in the next chapter, was found to be erroneous.

Nuttall[2] showed that the defibrinated blood of refractory sheep acted as a nutrient medium for the anthrax bacillus. Making comparative investigations, by the plate method, on the bactericidal

[1] *Ann. de l'Inst. Pasteur*, Paris, 1887, t. I, p. 42.
[2] *Ztschr. f. Hyg.*, Leipzig, 1888, Bd. IV, S. 353.

power of the blood of vaccinated and normal sheep, he observed that, in both cases, there was, at first, a certain decrease in the number of bacilli sown, more marked in the blood of the vaccinated than in that of the control animals. Nevertheless, 8 hours after the commencement of the experiment the anthrax bacteria had produced innumerable bacilli in the blood of the refractory sheep. Nuttall satisfied himself that this feeble bactericidal power was not to be compared with the very much greater power of the blood of the rabbit, an animal specially susceptible to anthrax.

More recently the properties of the serum of sheep which have been vaccinated against anthrax have been studied very carefully by Sobernheim[1]. He also was able to show that this serum allows of an abundant development of the bacillus, and that, outside the animal, it does not exercise any more appreciable bactericidal power than does the serum of the normal sheep. The serum of the best vaccinated sheep was found to be incapable of destroying even very small quantities of anthrax bacilli. The only change that Sobernheim could make out was with regard to the thickening of the bacterial membrane. This modification, however, was not constant and could not be seen in the serum of certain vaccinated sheep.

The serum of the sheep vaccinated by Sobernheim exhibited no
[255] increase of agglutinative power as regards virulent bacilli. Gengou[2], however, made it clear that repeated injections of cultures of the first vaccine of Pasteur into dogs produced a marked augmentation of this agglutinative power; but it was only produced when the attenuated bacillus was used. The virulent anthrax bacillus, developed as isolated rods, was not affected in the least by serum that was highly agglutinative for the bacillus of the first vaccine. Gengou also made the converse experiment with the serum of a dog into which he had previously injected a number of virulent anthrax bacilli. The dog, naturally refractory to anthrax, resisted the inoculation perfectly, but its serum did not acquire any agglutinative power against the first vaccine. He concluded therefrom that "the part played by agglutinins in the defence of the animal must be regarded as extremely problematical" (p. 339). On the other hand the phagocytic reaction in the vaccinated sheep is always very pronounced and constant. Von Behring[3], in one of his

[1] *Ztschr. f. Hyg.*, Leipzig, 1899, Bd. xxxi, S. 89.

[2] *Arch. internat. de Pharmacodyn.*, Gand et Paris, 1899, Vol. vi, pp. 303, 338.

[3] "Infectionsschutz und Immunität" in Eulenburg's "Real-Encyclopädie d. ges. Heilkunde," IIIte Aufl. (*Encyclop. Jahrbücher*), Wien, 1900, Bd. ix, S. 202.

most recent publications, expresses the opinion that this example of acquired immunity must be placed in the category of phagocytic immunity.

In the group of bacilli, several examples of which we have studied, the typhoid bacillus approaches still more closely to the vibrios and spirilla in its relation to humoral properties. Here may be observed a kind of attenuated Pfeiffer's phenomenon and somewhat profound modifications taking place under the influence of the serum of vaccinated animals. The *Bacillus pyocyaneus* is more resistant to the injurious influence of fluids taken from immunised animals. This resistance is still more marked in the bacillus of swine erysipelas and again still greater in the anthrax bacillus. Whilst, however, these properties of the fluids of the body are found to be very variable and of unequal power, the phagocytic reaction is constantly manifested and always very actively. The leucocytes which, in susceptible animals, exhibit a very marked negative chemiotaxis or only a tardy and incomplete positive chemiotaxis, have, in the vaccinated animal, this positive susceptibility developed in a very high degree.

Before quitting the group of bacteria we must cast a glance at the mechanism of acquired immunity against representatives of the [256] group of spherical micro-organisms. Amongst the cocci the streptococci have been especially studied as regards this immunity. For long great difficulties were encountered in vaccinating animals against these chain cocci, but Roger[1], Marmorek[2], Denys and Leclef[3] overcame these obstacles and succeeded in immunising the rabbit, one of the most susceptible species, to their pathogenic action. More recently the larger mammals, notably the horse, have been successfully immunised. A certain number of important facts, the knowledge of which is useful to complete the survey of the phenomena of acquired immunity, have thus been collected.

Roger set himself to study the properties of the blood serum of rabbits vaccinated against the streptococcus, and established the fact that this fluid had not the slightest appreciable bactericidal action; the streptococcus grew in it just as well as in the serum

[1] *Compt. rend. Soc. de biol.*, Paris, 1891, p. 538; 1895, pp. 124, 224; *Rev. de méd.*, Paris, 1892.

[2] *Ann. de l'Inst. Pasteur*, Paris, 1895, t. IX, p. 593.

[3] *La Cellule*, Lierre et Louvain, 1895, t. XI, p. 175; *Bull. Acad. roy. de méd. de Belg.*, Bruxelles, 1895, No. 11.

of fresh unvaccinated rabbits. When, however, he injected cultures grown in the serum of immunised animals into rabbits, these rabbits did not die and presented only transient and insignificant lesions. From this fact Roger concluded that there must be an attenuation of the streptococcus by the immune serum, a view which was shared by several other observers. In formulating this view, however, he had not taken into account the possibility that this serum acted not upon the coccus that had developed in it but upon the organism of the animal into which it was injected. Bordet[1], indeed, was able to show that the streptococcus which grows in the serum of immunised animals is in no way weakened in virulence. When he took a race very virulent for the rabbit (Marmorek's streptococcus) and injected a minimal dose of a culture grown in the serum of immunised animals, the rabbits died just as did the control animals, because the amount of serum introduced was too small to exert any influence. So also, when he filtered this culture and got rid of the serum bathing the streptococci, it was found to be just as
[257] virulent as that grown in the serum of susceptible unvaccinated animals.

In confirmation of the discovery made by Roger with the serum of vaccinated rabbits, Bordet showed that the blood serum of horses highly immunised against the streptococcus did not exhibit any bactericidal action. Moreover, he found that this serum caused the development of somewhat agglutinated streptococci and that it was capable of throwing streptococci grown on the ordinary media into clumps. Summing up his researches on the properties of this serum Bordet concludes that it "causes no profound change in the streptococcus. The vegetative character of the coccus is not appreciably diminished, and its morphology remains the same save for certain variations in the length of the chains. Even the agglutinative power, recognised in numerous serums by recent researches, is, in the antistreptococcic serum, developed but slightly" (p. 196).

More recently von Lingelsheim[2] has studied the properties of the serum of animals which he had thoroughly vaccinated against the streptococcus. He observed a certain slowing of the development of the coccus in this serum as compared with the growth in cultures made in the serum of normal, susceptible animals. But this re-

[1] *Ann. de l'Inst. Pasteur*, Paris, 1897, t. XI, p. 194.

[2] *Arch. internat. de Pharmacodyn.*, Gand et Paris, 1899, Vol. VI, p. 73; Behring's "Beitr. z. experim. Therapie," 1899, Bd. I.

tardation was slight and transient, and exhibited itself especially in serums to which von Lingelsheim, following Denys, had added leucocytes.

Von Lingelsheim also noted a certain degree of agglutination of the streptococcus by the serum of vaccinated animals, although this was much more feeble than in the case of the cholera vibrio or the typhoid bacillus, when agglutinated by their corresponding serums. Speaking generally, he regarded the direct action of the body fluids as insufficient to bring about the rapid destruction of the streptococci in the vaccinated organism. "Since the action of the bactericidal substances is limited in time, the streptococci are able to adapt themselves to these substances and recover their former energy. As the phenomena of extracellular solution, of such a form as those observed under the influence of the cholera antibodies, are absent in the case of the streptococcus and as, on the other hand, a considerable ingestion of these organisms by the leucocytes is observed.....we must seek in the activity of these cells a second important element of the defence of the animal organism" [258] (p. 78).

To Salimbeni[1], who has carried out in my laboratory an investigation on this subject, we are indebted for the most reliable information on the phagocytic reaction in acquired immunity against the streptococcus. He studied specially the phenomena in the subcutaneous tissue of a horse, hypervaccinated against Marmorek's streptococcus; this animal received in all, at several injections, about five litres of living culture. In spite of this refractory condition, an oedema at the point of inoculation was soon produced; in this the micro-organisms remained free and the leucocytes were sparse. But the cellular reaction, at first insignificant, developed with great rapidity and many leucocytes, amongst which the macrophages were much the more numerous, were attracted. The phagocytosis was still delayed for some time, but it continued to increase and 20 to 24 hours after the inoculation it was complete. As soon as the phagocytosis was well established the oedema began to disappear. In the thick exudation, containing a mass of leucocytes, the macrophages are filled with a very large number of streptococci packed together. These cocci develop inside the cells, cause them to burst and again become free. A fresh arrival of leucocytes, however, takes place, this time mainly microphages.

[1] *Ann. de l'Inst. Pasteur*, Paris, 1898, t. XII, p. 192.

These microphages seize the free streptococci that have struggled so victoriously against the macrophages; this second phagocytic phase is final. The streptococci still remain alive inside the microphages for some days, but ultimately are killed and digested by the phagocytes. At a period when, 5 or 6 days after injection, insignificant or isolated traces of streptococci are to be found in the microphages, the exudation when sown in nutritive media still gives abundant cultures. The incidents of this struggle between the streptococcus and the animal organism demonstrate the important part played by the phagocytes. The fact that the macrophages perish and allow the cocci to escape, clearly proves that these cocci have been ingested alive and virulent, and consequently that the fluid of the exudation was incapable of destroying or even of attenuating them. The macrophages, also, were powerless to bring
[259] about this result and the intervention of the microphages was necessary to cause the disappearance of the cocci. It is, however, always the phagocytes which ensure the final resistance of the animal.

In presence of these very precise results obtained from the work of Salimbeni, a work which I followed very closely, the previous researches by Denys and Leclef (*l.c.*) made under less favourable conditions on vaccinated rabbits are deprived of their importance. These observers wished to get an idea of the difference between the reactions of the animal organism (*a*) after the injection of streptococci into the pleural cavity of immunised rabbits, and (*b*) after injection into that of normal susceptible rabbits. They killed the inoculated animals and found a very marked diminution of micro-organisms in the pleuritic exudation of the former. This diminution could not be attributed to a lysis of the streptococci by the body fluids, because there were never any signs of such destruction. Nor could the phagocytosis, very feeble at first, be considered as the cause of the disappearance of a large number of the streptococci. Denys and Leclef put forward a third hypothesis, which attributed this disappearance to the rapid resorption by the lymph stream of the injected fluid containing the organisms. Going over the record of their experiments it will be seen that in vaccinated rabbits the quantity of pleuritic exudation was always very much less than in normal rabbits. In presence of this feature there is reason to ask whether, in the case of the streptococci, a large number of these organisms were not fixed, along with the leucocytes, on the walls of

the pleura, as in guinea-pigs that are inoculated intra-peritoneally? Instead of being satisfied with merely examining the fluid exudation, the surface of the pleura should have been scraped in order to ascertain whether the phagocytic reaction was localised in this region.

In any case such incomplete results on the active immunity of rabbits in no way weaken the positive results obtained in the subcutaneous tissue of the horse, in which the phagocytic reaction plays a really preponderant part.

This example of the streptococci completes our series of bacteria in which we have studied their relations with the properties of the animal organism that has acquired immunity. We have still to see whether the acquired immunity against micro-organisms of animal origin is subject to the same law as that against bacteria.

For some years past a zealous study of the infectious diseases pro- [260]
duced by animal micro-organisms has been carried out. Besides malaria, which occupies a most important position, attention has been directed to certain diseases in domestic animals that are set up by endoglobular haematozoa and by flagellata, and a fairly large number of accurate data have been collected with regard to Texas fever and its parasite the *Piroplasma bigeminum*, as well as upon the epizootic diseases due to *Trypanosomata* (Tsetse fly disease or Nagana, "Dourine," etc.).

We are indebted to Smith and Kilborne[1] for the earliest information concerning the acquired immunity of Bovidae against Texas fever. R. Koch[2] then added some very precise observations on the immunity of calves which had been inoculated with parasites attenuated in the body of the tick (*Boophilus bovis*). Lignières[3], who devoted much attention to this question in the Argentine Republic, has discovered a sure method of vaccinating the Bovidae against the "Tristeza," the local name for Texas fever. He brought to Alfort specimens of attenuated haematozoa, and in Nocard's presence performed successful vaccination experiments. Lignières is now engaged in devising a practical method of ensuring immunity under the special conditions found in the home of the "Tristeza." Up to the present, however, there are no sufficient data as to the mechanism of

[1] *Bulletin No.* 1, *Bureau of Animal Industry, U.S. Dep. of Agric.*, Washington, 1893.

[2] "Reisebericht über Rinderpest etc.," Berlin, 1898.

[3] *Rec. de méd. vét.*, Paris, juillet, 1900, and *Ann. de l'Inst. Pasteur*, Paris, 1901, t. xv, p. 121.

the acquired immunity in this case. We have fuller information as to the essential phenomena observed in the organism of the rat vaccinated against *Trypanosoma lewisi*. We owe to Mme. L. Rabinowitsch and Dr Kempner[1] the first important data as to the possibility of immunising white or piebald rats against the disease produced by the flagellated infusorian. They noted that these animals when inoculated with the blood of grey rats containing *Trypanosomata* acquire a very transitory disease which, however, confers an immunity against any subsequent infection. The flagellated organisms disappear from the blood within a few weeks, after which fresh injections of these parasites have no pathogenic effect.

[261] Laveran and Mesnil[2] confirmed these observations, and in addition made careful observations on the mechanism of this acquired immunity. After making several inoculations with blood containing *Trypanosomata* into white rats, they made a study of the properties of the blood serum of these immunised animals. First they established the fact that this serum exerts no microbicidal action on the *Trypanosomata*, but it agglutinates them without, however, rendering them motionless:—"The masses may be resolved into rosettes in which the *Trypanosomata*, united merely by their posterior extremities, have their flagella free and motile at the periphery."

Laveran and Mesnil then studied the phenomena evolved in the refractory organism. When injected into the peritoneal cavity of immunised rats the *Trypanosomata* are not acted upon injuriously by the body fluids. They are, however, devoured by the leucocytes. Laveran and Mesnil thus express themselves on this subject: "...we have demonstrated clearly and repeatedly that the *Trypanosomata* are ingested alive, perfectly isolated and very motile, by phagocytes, and we have followed the details of this process of ingestion which recalls that of the ingestion of spirilla by the leucocytes of the guinea-pig. We consider, therefore, that the immunity is phagocytic in character."

The main facts on acquired immunity established in connection with the most diverse micro-organisms, facts just described, may already be said to lead to certain general conclusions. They indicate in the first place that acquired immunity is accompanied by phenomena more complicated than those observed in natural immunity. In the two categories of processes observed in acquired immunity the pha-

[1] *Ztschr. f. Hyg.*, Leipzig, 1899, Bd. xxx, S. 251.
[2] *Ann. de l'Inst. Pasteur*, Paris, 1901, t. xv, p. 673.

gocytic reaction is the only one that can be said to be constant. We find it in those examples in which the influence of the fluids of the body is most manifest, as in the experimental cholera peritonitis of the guinea-pig, as well as in those cases where the humoral action is most feeble, as in anthrax or in the *Trypanosoma* disease of rats. We have, however, still to establish the relations that exist between phagocytosis and the part played by the fluids of the immunised animal, in order that we may, as far as possible, present a general picture of the inner mechanism of acquired immunity against micro-organisms. To attain this result we must place the reader in posses- [262]
sion of further well-established facts, and we must postpone its discussion to the following chapter, which will be entirely devoted to the above-mentioned problem.

[263]

CHAPTER IX

THE MECHANISM OF ACQUIRED IMMUNITY AGAINST MICRO-ORGANISMS

Cytases and fixatives.—Only the latter are augmented in the immunised organism.—Properties of the fixatives.—Difference between them and the agglutinative substances.—The part played by the latter in acquired immunity.—Protective property of the fluids of the immunised organism.—Stimulant action of the body fluids.—The protective power of serum cannot serve as a measure of acquired immunity.—Examples of acquired immunity in which the serums exhibit no protective power.—Phagocytosis in acquired immunity.—Negative chemiotaxis of leucocytes.—Theory of attenuation of micro-organisms by the fluids of immunised animals.—Refutation of this theory.—Phagocytosis acts without requiring any previous neutralisation of the toxins.—The origin of the fixative and protective properties of the body fluids.—The relation between these properties and phagocytosis.—The side-chain theory of Ehrlich and the theory of phagocytes.

Whilst, in natural immunity against micro-organisms, humoral phenomena play no prominent part, in acquired immunity these phenomena assume a much greater importance. The bactericidal power of the fluids of the body is, in natural immunity, reduced to a mere trace, for it has been demonstrated that the power of normal serums to destroy bacteria corresponds to no natural phenomenon of the living organism, but is dependent upon the presence of cytases which have escaped from the phagocytes at the time of the formation of the clot *in vitro* and separation of the serum. The presence of the fixative, that other important element in immunity, has been demonstrated in the normal fluids only in rare cases and in small quantity. The agglutinative property of these fluids has likewise shown itself to be little developed and without any importance in natural immunity.

In acquired immunity against micro-organisms, on the other hand, we find that the bactericidal and agglutinative powers of the

fluids of the body are very greatly increased. With the discovery that the bactericidal property was so highly developed in the serums of animals that had been vaccinated against vibrios arose the belief in [264] the acquisition of a new and purely humoral property. R. Pfeiffer, especially, insisted on the fundamental difference between the power of the serum of immunised animals to transform the cholera vibrios into granules and the corresponding property of normal serums. In the first case Pfeiffer's phenomenon exhibited marked specificity ; in the second, it was much more general. A normal serum transforms into granules, indifferently, vibrios that are very distinct from one another ; whilst the serum of an animal vaccinated against a particular species or race of vibrios gives Pfeiffer's phenomenon with this species or race only. Bordet's[1] researches have definitely settled this question. This investigator has shown that Pfeiffer's phenomenon is produced, with all the usual serums, by means of the same substances, the cytases (alexine, or complement of Ehrlich). But in the serum of vaccinated animals there is added to these cytases the fixative (sensibilising substance of Bordet, immunising body or amboceptor of Ehrlich) which exhibits specific properties. Having thus carefully distinguished the two substances that set up the granular change in vibrios, Bordet shows that in vaccinated animals it is the fixative which increases in quantity, whilst the cytase remains pretty much in the same proportions as in the normal animal. He demonstrated, in fact, that when we take a very small dose of the serum of a vaccinated animal which by itself is incapable of transforming the vibrios into granules, about the same quantity of immunised serum or of normal serum must be added to it in order that Pfeiffer's phenomenon may appear. The quantity of cytase, that soluble ferment which is necessary for the production of the phenomenon, is, therefore, about the same in the serum of a normal animal as in that of a well-vaccinated animal. Whilst the cytase does not increase as a result of vaccinal injections, the fixative, on the other hand, becomes more and more abundant. Consequently it is this second soluble ferment that impresses its characters on the blood serum and on some of the other fluids of the vaccinated animal. It has been pointed out in the preceding chapter that the fixative is found in the fluid of the oedema of vaccinated animals, although in less quantity than in their blood serum. It has also been mentioned that no fixative is found in the aqueous humour of well-vaccinated [265]

[1] *Ann. de l'Inst. Pasteur*, Paris, 1895, t. IX, p. 462.

animals. It must be admitted that this ferment is not inseparably bound to the cells which produce it, as is the case with the cytases. I have already developed, at some length, the thesis that the cytases remain, in the normal animal, within the phagocytes, and only escape from them when these cells are destroyed, whether in the living animal (during phagolysis) or outside the animal (during the preparation of the serum). Gengou's experiments with the plasma and the blood serum of normal animals have completely confirmed the fundamental observations that the cytases are not found free in the circulating blood. It is evident that the same law applies also to an animal that has acquired immunity. For this reason neither Pfeiffer's phenomenon nor any analogous process that demands the action of cytases is ever produced in the anterior chamber of the eye, or in the subcutaneous tissue, or in oedema either active or passive. Further, it is in virtue of this same law that Pfeiffer's phenomenon does not manifest itself even in the peritoneal cavity or in the blood vessels of vaccinated animals in which the phagocytes have been protected from phagolysis by previous injections of various fluids (physiological saline solution, broth, etc.). It would be very interesting to be able to demonstrate the absence of cytases in the fluids of immunised animals by experiments of the same order as those carried out by Gengou with the fluids of normal animals, but the obstacles to the realisation of this postulate are too great. We saw when discussing Gengou's experiments that it is impossible to obtain *in vitro* a fluid identical with the plasma of living blood. The greatest precautions in collecting the blood and in its after treatment are insufficient to prevent coagulation taking place sooner or later. It follows that, as there is always a considerable quantity of free fixative in the plasma of immunised animals, an infinitesimal quantity of microcytase, set free from the leucocytes, is sufficient for the production of Pfeiffer's or any other analogous phenomenon. There must be a great improvement in the methods of preparation of plasmas outside the body before it will be possible to undertake successful researches on the above problem. For the present we must rest satisfied with other proofs, already numerous and very demonstrative, of the absence of free cytases in the normal plasmas of vaccinated animals.

[266] The cytases being found in about the same quantity and presenting the same properties in all animals that enjoy immunity whether natural or acquired, it must be the fixative which specially

distinguishes these two categories of immunity. Now, the fixative is found in the serum of perhaps all cases of acquired immunity. Bordet and Gengou have studied it by the method already mentioned (Chap. VII.). A certain quantity of micro-organisms of various species is introduced into the serum. If the cytases, present in the serum when the experiment was commenced, ultimately disappear from it, it indicates that this ferment has been absorbed by the bacteria, thanks to the fixative, which consequently should be found in the serum under observation. The presence or absence of the cytases can be demonstrated by the production or absence of Pfeiffer's phenomenon with vibrios.

The application of this method enabled Bordet and Gengou[1] to satisfy themselves that the serum of animals immunised against several species of bacteria (plague bacillus, typhoid bacillus, bacillus of swine erysipelas, first anthrax vaccine, and *Proteus vulgaris*), really contains an appreciable quantity of fixative. It may, then, be accepted that the production of this substance is fairly constant in acquired immunity against bacteria, and that it constitutes one of the most important factors in such immunity.

The question has been raised: What is the nature of the substance to which the name of fixative is given? Pfeiffer and Proskauer[2] have attempted to solve this question by making use of a serum which acts against the cholera vibrio and which they obtained by vaccinating animals with this vibrio. They carried out a long series of experiments which led them to the conclusion that this substance, which they term "cholera antibody," cannot be identified with any of the albuminoid substances of the serum. Further, the fixative is not represented by any of the salts or extractive substances of the serum, because these substances dialyse easily, whereas the cholera antibody does not pass through the dialysing membrane. The fixative is wholly precipitated by alcohol, and is regarded by Pfeiffer and Proskauer as belonging to the category of soluble ferments, an opinion which is certainly shared by many other investigators.

What is it that communicates to this ferment its remarkably [267]
specific character? Without being able to give a precise answer to this question, the authors just cited point out the analogy that exists between the cholera antibody and the soluble ferments of yeasts which have been studied by Emil Fischer. Some of these

[1] *Ann. de l'Inst. Pasteur*, Paris, 1901, t. xv, p. 289.

[2] *Centralbl. f. Bacteriol. u. Parasitenk.*, Jena, 1896, 1te Abt., Bd. xix, S. 191.

act only upon certain special sugars in a manner equally specific. From a logical point of view it might be permissible to attribute the specificity of fixatives to something borrowed from the species of micro-organism that has played a part in their production. It has long been recognised that in old cultures of the cholera vibrio these micro-organisms are transformed into spherical granules, the arthrospores of Hueppe, which closely resemble the granules produced in Pfeiffer's phenomenon. There are, then, undoubtedly, vibrionic products which act much as do the microcytases, and it would be very interesting if we could find them in the bactericidal ferments of the animal body. An attempt of this kind was undertaken by Emmerich and Löw[1], who attribute the acquired immunity to a particular substance which they term "Nuclease-Immunproteïdin." According to their hypothesis the microbial products which are produced in the animal during the period of vaccination—the nucleases—combine with proteid substances of the blood and organs to furnish the substance to which these authors have given such an elaborate name. In their most recent publication Emmerich and Löw even describe a method of producing this substance outside the animal body, by the action of ox blood, or better still pounded spleen, on the nuclease produced by the bacteria found in old cultures. To it they attribute the property of dissolving the various bacteria, of conferring immunity against and even of curing several infective diseases. But these authors do not say whether this remarkable substance is identical with, or analogous to, the antimicrobial ferments composed, as we have seen, of microcytase and fixative. It must be concluded that they look upon it as being similar to the alexine of Buchner, which is nothing more than a mixture of the two substances just named. Unfortunately the whole account given by Emmerich and Löw will do anything but gain over the reader, and in their publications no proof of their assertions can be found. Several of the facts advanced by them do not fall in with well-established data. Thus they speak
[268] of the complete lysis of the bacilli of swine erysipelas by their soluble "Erysipelase-Immunproteïdin" in vaccinated animals, a process that has never been demonstrated by them and which in no way accords with conscientious and carefully carried out observations. On the other hand, they cite facts which contradict one another. The "Pyocyanase-Immunproteïdin" is a substance which possesses an extraordinary bactericidal power, not only against the *Bacillus pyo-*

[1] *Ztschr. f. Hyg.*, Leipzig, 1901, Bd. xxxvi, S. 9.

cyaneus but also against several other bacteria, *e.g.* the bacilli of anthrax, diphtheria, typhoid, and plague. This substance rapidly breaks up these bacteria, and cures diphtheria and experimental anthrax. But it is, at the same time, so affected by the invasion of the most common bacteria, such as *Bacillus subtilis*, that it is necessary to add antiseptics in order to preserve it. To these contradictions, inaccuracies, and uncertainties must be added further the advice, given by Emmerich and Löw to bacteriologists, not to attempt to reproduce their experiments, because they may easily fail, and I think that, in spite of the seductiveness of the attempt to attribute to bacterial products a share in the elaboration of antimicrobial substances, we must conclude not to follow these authors further. It is better to confess our ignorance of the chemical composition of these substances in general and of the fixatives in particular.

As the fixatives resist temperatures much higher than those which destroy the cytases, in this respect resembling the agglutinative substances so frequently found in the fluids of vaccinated animals, there has long been a tendency to identify them with these latter. It is indisputable that between the fixatives and the agglutinative substances the analogies are fairly numerous. Both are produced in quantity during the process of immunisation, and are found not only in the blood serum but also in the fluids of the living animal, especially in the fluids of exudations and transudations. Both dialyse through parchment more readily than do the cytases. Buchner[1] has demonstrated that his alexines (bactericidal substances of normal serum) will dialyse only where the lower fluid is pure water; dialysis is *nil* when the distilled water is replaced by physiological saline solution. The fixatives and agglutinins, as demonstrated by Gengou[2] for the [269] latter, pass almost completely through the dialyser in the case of pure water, and one-half still passes when the lower fluid approaches as nearly as possible to normal serum.

In spite of these analogies, however, the agglutinative property must be sharply distinguished from the fixative power of serums. In this fluid, derived from normal animals, the agglutinative property is often very marked when the power of fixing the cytases is totally, or in great part absent. Bordet and Gengou[3] have demonstrated also

[1] *München. med. Wchnschr.*, 1892, SS. 119, 982.
[2] *Ann. de l'Inst. Pasteur*, Paris, 1899, t. XIII, p. 647.
[3] *Ann. de l'Inst. Pasteur*, Paris, 1901, t. XV, p. 289.

that feebly agglutinative serums of persons convalescent from typhoid fever may exhibit a great capacity for fixing the cytases. Other facts, to be mentioned later, confirm the real difference between the fixative and the agglutinative properties.

The agglutination of bacteria was noted during the course of a series of researches on the acquired properties of the blood serum of vaccinated animals. Charrin and Roger[1], seeking to obtain a clear idea of the difference between the serum of normal animals and that of animals vaccinated against the *Bacillus pyocyaneus*, observed that this bacillus developed in the normal fashion in the former, but in the latter gave rise to special forms of growth. Instead of growing in the form of rods, it elongates into segmented filaments which become entangled and fall to the bottom of the tubes, leaving a supernatant limpid serum. I was able not only to confirm the accuracy of this observation for the *Bacillus pyocyaneus*, but to extend it to Gamaleia's vibrio and to the pneumococcus[2]. In all these instances we have a modification of the bacteria developed in specific serums coming from vaccinated animals. Later, Bordet[3], during his researches on the bacteriolysis of vibrios *in vitro*, observed that these vibrios, when introduced into the blood serum of vaccinated animals, lose their movements and soon unite into more or less voluminous masses. This observation was confirmed by Gruber
[270] and Durham[4], who were the first to apply it in the specific diagnosis of bacteria. They showed that the agglutinating power of vaccinated animals, although not rigorously specific, might, nevertheless, be utilised for the differentiation of certain bacteria, especially the cholera vibrio and the typhoid bacillus. But, independently of this result, Gruber[5] essayed to formulate a theory of acquired immunity based on the agglutinative property of the serum. He accepted, in connection with the phenomenon of the destruction of the bacteria, Bordet's hypothesis of the concurrent action of two substances, of which one, the bactericidal substance proper, is nothing but the alexine of Buchner, the second being that which agglutinates the bacteria. This agglutination, according to Gruber, results from

[1] *Compt. Rend. Soc. de Biol.*, Paris, 1889, p. 667.

[2] *Ann. de l'Inst. Pasteur*, Paris, 1891, t. v, p. 473.

[3] *Ann. de l'Inst. Pasteur*, Paris, 1895, t. ix, p. 462.

[4] *München. med. Wchnschr.*, 1896, S. 285 [cf. also Durham, *Journ. Path. and Bacteriol.*, Edin. and London, 1897, Vol. iv, p. 13, and 1901, Vol. vii, p. 240; *Brit. Med. Journ.*, London, 1898, Vol. ii, p. 588].

[5] *Wien. klin. Wchnschr.*, 1896, SS. 183, 204.

the swelling of the bacterial membrane which becomes viscous and so leads to the cohesion of the bacteria and the formation of clumps. Thus transformed and rendered motionless, the bacteria succumb more readily to the destructive action of the alexine. It is supposed that the phagocytes do not intervene at all in these cases of acquired immunity, except in a purely secondary fashion when they ingest the bacteria already greatly weakened by the united action of the agglutinin and the alexine. The principal *rôle* in this theory of immunity is thus given to the agglutinative substance, which is regarded as being a microbial product, modified by the macrophages and thrown into the blood.

The discovery of this agglutination of bacteria has acquired great importance, especially in connection with its application to the diagnosis of typhoid fever. Widal[1] succeeded in showing that typhoid bacilli agglutinate readily under the influence of blood serum and other fluids (milk, transudations, tears, etc.) derived from patients suffering from typhoid fever. As this phenomenon could be utilised for the early recognition of the disease, it began to be studied with great care and many interesting data concerning it have been collected. The general outcome of these researches accords with the conclusions drawn by Widal, and the serum-diagnosis of typhoid fever has taken an important place among the methods used for the recognition of this disease. This aspect of the question, however, does not interest us from the point of view of the problem of immunity which we now have under consideration, and we cannot here enter upon the study of the serum-diagnosis of typhoid fever and certain other diseases (cholera, tuberculosis, pneumonia). Moreover, [271]
we must refrain from any analysis of the hypotheses advanced to explain the mechanism of agglutination. A lively discussion has been carried on between the partisans of the chemical theory—according to whom the agglutinin acts directly on the agglutinable substance of the bacteria—and the advocates of the physical theory, led by Bordet[2], who attribute the agglutination to modifications in the molecular attractions which unite the agglutinable elements, be it between each other or with the surrounding fluid. At one time it was thought that Roger's[3] observation that the cell membranes of *Oïdium albicans*, when cultivated in the specific serum of

[1] *Bull. Soc. méd. d. hôp.*, Paris, 1896, 26 juin [*Semaine méd.*, Paris, 1896, p. 259].
[2] *Ann. de l'Inst. Pasteur*, Paris, 1899, t. XIII, p. 225.
[3] *Rev. gén. d. sc. pures et appliq.*, Paris, 1896, t. VII, p. 770.

immunised animals, increased in volume and became greatly swollen, settled the question in favour of Gruber's theory. But the objection formulated by Kraus and Seng[1], on the one hand, and by Bordet, on the other, dealt a severe blow to this view. As the serum employed by Roger was not deprived of its cytases (alexine), the viscosity of the membrane of the fungus could not be attributed to the agglutinin. When Bordet[2] demonstrated that the red blood corpuscles, under the influence of the serums, undergo an agglutination as marked as that seen in bacteria, it enabled us to study this phenomenon in the large red corpuscles of birds, in which no one has ever been able to demonstrate any viscosity of the corpuscular stroma. In a mixture of red corpuscles of bird and mammal, submitted to the action of a serum which agglutinates the former only, the red corpuscles of the mammal never unite with those of the bird, although this should undoubtedly take place if the membrane of the agglutinated corpuscles had really become viscous. All the facts collected up to the present are, therefore, in favour of Bordet's physical theory in which an analogy between the phenomena of agglutination and of coagulation is traced.

The point that interests us more particularly in regard to agglutination is the relation of this phenomenon to immunity. We have already given (Chapter VII) the arguments which render it impossible for us to attribute to the agglutinative property of the fluids of the body any *rôle*, however unimportant, in natural immunity against
[272] micro-organisms. We must now study the importance of this property in the condition of acquired immunity, in which the agglutination of micro-organisms by the fluids of the body is much more frequent and active than in natural immunity.

The first question to be settled is the following: Is the agglutinative property really constantly present in the fluids of vaccinated animals? The blood serum of animals that have acquired immunity is unquestionably usually agglutinative as regards the corresponding micro-organism. This agglutination may be more or less pronounced, but it certainly exists in the great majority of cases. Nevertheless, examples can be cited in which, in spite of the refractory condition acquired as the result of immunisation, the serum exhibits not a trace of agglutinative power. Having demonstrated that several bacteria (*Bacillus pyocyaneus, Diplococcus pneumoniae, Vibrio*

[1] *Wien. klin. Wchnschr.*, 1899, S. 1.
[2] *Ann. de l'Inst. Pasteur*, Paris, 1898, t. XII, p. 688.

metchnikovi) develop in the serum of vaccinated animals in the form of elongated filaments more or less interlaced, I was quite prepared to allow that this fact might be of general import. But the study of a cocco-bacillus which produces the pneumo-enteritis of swine and which was isolated by Chantemesse during an epizootic at Gentilly, led me to believe that this was not the case. As this bacillus is characterised by great motility, I concluded[1] that it was identical with that of the hog cholera of American writers. Theobald Smith[2], to whom I sent a specimen and who is a competent authority on this question, refers it, however, to the species which produces swine plague. Knowing that the question of these two bacteria is not finally settled, it is impossible to come to an absolute decision in the matter. Fortunately, from the point of view of immunity, this is of no great importance. The point upon which I must lay stress is that the serum of rabbits vaccinated against the Gentilly bacillus, when sown with this cocco-bacillus, gave very abundant and uniformly turbid growths. In my researches, undertaken at a period when the rapid agglutination of micro-organisms added directly to the specific serum had not yet been recognised, I noted merely that the cocco-bacilli which grew in the blood serum of vaccinated rabbits presented their normal form and gave rise to a general turbidity of the fluid. Since then, however, it has often been observed that the mode of development of a micro-organism in a serum gives an even more delicate indication than does the agglutination properly so called, produced by the serum to which [273] has been added an organism cultivated on its usual medium. Thus Pfaundler[3] saw that *Bacillus coli* and *Proteus vulgaris*, which were not agglutinated by certain serums, developed in them in an unusual fashion and produced very long and interlacing filaments. When a serum is incapable of revealing its properties by agglutinative reaction properly so called, it is sown with the corresponding micro-organism and the development is then compared with that observed in a normal serum. Frequently a very marked difference is noted, the same organism growing into filaments in the specific serum and forming rods only in the normal serum. The first mode of development is sometimes designated "Pfaundler's reaction."

[1] *Ann. de l'Inst. Pasteur*, Paris, 1892, t. VI, p. 289.

[2] *Centralbl. f. Bakteriol. u. Parasitenk.*, Jena, 1894, Bd. XVI, S. 235.

[3] *Centralbl. f. Bakteriol. u. Parasitenk.*, Jena, I^te^ Abt., 1898, Bd. XXIII, SS. 9, 71, 131.

In the serum of rabbits vaccinated against the Gentilly cocco-bacillus, no filaments corresponding to those met with in the agglutinative reaction are formed, but bacilli are produced. In spite of this the animals that furnish the serum show a distinct resistance to infection. More recently, Karlinski[1] has studied the properties of the serums of animals treated with the cocco-bacilli of hog cholera and swine plague. He was able to demonstrate that blood serum from oxen that had received repeated injections of cultures or toxin of hog cholera, was not only incapable of killing the cocco-bacilli of the two swine diseases but it even "gave rise to no agglutination" of the two bacilli and did not arrest the motions of those of hog cholera. On the other hand, serums have been obtained from other species of animals (dog, pig) which brought about the typical agglutination of the cocco-bacillus of hog cholera[2].

In the preceding chapter, Gengou's experiment on the serum of a dog that had been treated with a virulent culture of anthrax has already been cited. This serum did not agglutinate the bacillus, even of the first vaccine of Pasteur. Nevertheless, a second dog treated with an attenuated culture of this bacillus furnished an agglutinative serum. The immunisation of the first dog was carried very much further than that of the second, but the agglutinative properties were in inverse order. Sawtchenko, in his study of immunity against anthrax, demonstrated that the subcutaneous exu-
[274] dation from vaccinated rats does not agglutinate the bacillus which usually exhibits such a great tendency to collect into clumps.

Agglutination has been studied particularly carefully in typhoid fever. We know that after an attack of this disease, an acquired refractory condition is produced which lasts for a considerable period. In most cases the agglutinative power of the blood diminishes very rapidly, and disappears a few weeks after the commencement of convalescence. It is only in rare cases that it persists for years[3]. On the other hand, during the period of apyrexia which precedes the relapse in typhoid fever and during the period of relapse, the agglutinative power may manifest itself in a very marked degree. In an observation made on a case reported by Widal and

[1] *Ztschr. f. Hyg.*, Leipzig, 1898, Bd. XXVIII, S. 406.

[2] *Fifteenth Ann. Rep. of the Bureau of Animal Industry for* 1898, Washington, 1899, p. 348, Pl. XI.

[3] Widal et Sicard, *Bull. et Mém. Soc. méd. d. hôp.*, Paris, 1896, p. 684 [*Semaine méd.*, Paris, 1896, p. 514].

Sicard[1], the agglutinative power was raised, two days before the relapse, to a ratio (1 : 150) it had never attained during the first attack. "The appearance of the relapse, two days after this observation"—these authors add—"renders it evident that the agglutinating reaction is independent of the state of immunisation." Analogous cases have been pointed out repeatedly by several observers.

The examples cited show, on the one hand, that the serum of individuals endowed with acquired immunity may be without any agglutinative property, but, on the other, that this power may be highly developed in the serum of susceptible individuals. The argument based on these data may be corroborated by several other series of facts. Thus, Salimbeni[2] has pointed out that the cholera vibrio is not agglutinated in the fluids of immunised animals. The subcutaneous exudation of a horse treated with a large quantity of these vibrios does not agglutinate Koch's vibrio except outside the body. When this exudation is drawn off shortly after the injection of the vibrios, the organisms render the fluid uniformly turbid. But a short exposure to the air is sufficient to bring about the agglutination of the vibrios in the same exudation. Guided by this observation, Salimbeni carried out comparative experiments on the action of the serum of vaccinated animals outside the body, in tubes deprived of oxygen and in others exposed to the air. In the former agglutination did not take place or was very incomplete, in the latter it [275] soon came on. This fact accords perfectly with the observation of Pfeiffer's phenomenon in the peritoneal cavity of guinea-pigs from which we withdraw a fluid containing granules that have resulted from perfectly isolated vibrios. In other micro-organisms a difference has been noted in this respect. Thus Gheorghiewsky has seen the agglutination of the *Bacillus pyocyaneus* produced under the influence of the serum of vaccinated animals, even in tubes deprived of oxygen. Durham has made a similar observation in the case of the typhoid bacillus. When, however, Trumpp[3] wished to satisfy himself as to the agglutination of the same organism in the body of well-vaccinated guinea-pigs, he obtained only imperfect results. He concluded from his experiments "that the formation of typhoid clumps may precede the breaking down of the bacteria in the

[1] *Ann. de l'Inst. Pasteur*, Paris, 1897, t. XI, p. 411.
[2] *Ann. de l'Inst. Pasteur*, Paris, 1897, t. XI, p. 277.
[3] *Arch. f. Hyg.*, München u. Leipzig, 1898, Bd. XXXIII, S. 124.

animal body itself, but only under certain conditions—when the degree of immunity of the animal is sufficiently high and when the bacilli introduced are not too numerous" (p. 130). In the case of the typhoid bacillus, a certain degree of agglutination is produced inside the animal body, but it is markedly increased in the fluids that have been withdrawn and exposed to the action of the air.

It has been demonstrated, repeatedly, that the agglutination of micro-organisms by their specific serums does not prevent their growth and multiplication. These agglutinated organisms lose none of their virulence. Issaeff[1], working in my laboratory, carried out an investigation on this point in the case of the pneumococcus. He vaccinated rabbits against this organism and satisfied himself that the organism still grows well in the blood serum of such rabbits; but, instead of presenting the typical form of lanceolate diplococci, the pneumococcus, under these conditions, forms very long chains of true streptococci. Having filtered the cultures in order to get rid of the serum, he injected them into rabbits and mice and demonstrated that the pneumococci had retained to the full their initial virulence. Sanarelli[2] carried out corresponding experiments with Gamaleia's vibrio, which, as we know, also forms chains in the serum of vaccinated animals. When filtered on a paper filter and washed with physiological saline solution, the vibrios were found to be just as virulent as were the control vibrios grown in
[276] the serum of susceptible animals. More recently, Mesnil[3] demonstrated the same point in connection with the bacillus of swine erysipelas. He experimented on cultures that were agglutinated after their formation and also on others agglutinated as they were growing. The fluid of the culture was decanted and replaced by fresh broth until the elimination of the serum was complete. Mice, inoculated with the washed clumps, died in the normal period, thus affording proof that "agglutination in no way alters the vitality and virulence of the bacillus of swine erysipelas" (p. 492).

We can readily understand, after the demonstration of these various facts, that it is impossible to maintain Max Gruber's theory that the agglutinative power constitutes the fundamental basis of acquired immunity. Hence this writer, after publishing several preliminary notes in 1896, has not yet decided to give to his hypothesis

[1] *Ann. de l'Inst. Pasteur*, Paris, 1893, t. VII, p. 260.
[2] *Ann. de l'Inst. Pasteur*, Paris, 1893, t. VII, p. 225.
[3] *Ann. de l'Inst. Pasteur*, Paris, 1898, t. XII, p. 481.

a more extended development. Nor has any one else attempted to defend it.

It is probable that in certain special cases the immobilisation of very motile bacteria and their agglutination into clumps may facilitate the reaction of the animal organism, especially the rapidity of phagocytosis. Thus, Besredka[1] observed that guinea-pigs when inoculated with typhoid bacilli that had previously been mixed with the blood serum of normal animals survived. The most active amongst these serums was ox serum heated to 60° C. Guinea-pigs furnished a serum which was much less active. The resistance of guinea-pigs, inoculated into the peritoneal cavity, was in direct ratio to the agglutinated condition of the bacilli. Besredka lays stress on the facility with which the bacilli, when agglomerated into large clumps, were ingested by the phagocytes, and suggests that there is a certain stimulating action of the serums on the leucocytes. When he injected into guinea-pigs a mixture of typhoid bacilli and guinea-pig's serum, made immediately before injection, his animals died from infection. But when he left the bacilli for some time in contact with the guinea-pig's serum outside the body, and did not inject the mixture until after agglutination was complete, the inoculated animals usually survived. This experiment indicates the part played by agglutination in the resistance offered by the animal, and at the same time proves that in the body of the guinea-pig the agglomeration of the micro-organisms into clumps does not take place to the [277] same degree as in the serum prepared in, and left in contact with, the air.

In any case, the data collected by Besredka cannot be put forward as an argument in favour of the essential part played by agglutination in acquired immunity, nor can they weaken the facts indicated as to the absence of agglutinative power in examples of acquired immunity and as to the virulence of the agglutinated micro-organisms. The part played by agglutination in this immunity is merely accidental and subordinate.

Special researches have been carried out with the object of defining, exactly, the origin of agglutinins in the body of an animal that has acquired immunity. Observers are unanimous in recognising that, of all parts of the organism, the blood is richest in agglutinin. This substance is found in the blood serum as well as in the plasma. From this (corroborated by the agglutinative property of other

[1] *Ann. de l'Inst. Pasteur*, Paris, 1901, t. xv, p. 209.

fluids, such as the pericardial fluid, oedemas very poor in formed elements, etc.) it follows that the agglutinin circulates in the blood and lymph of the living animal. Several observers, amongst whom I may cite Achard and Bensaude[1], Arloing[2], and Widal and Sicard[3], put to themselves the question whether, before passing into the blood, the agglutinin is not formed in the exudation developed at the seat of inoculation of the micro-organisms. Their conclusions were invariably negative; they were never able to find more agglutinins in these exudations than in the blood. Pfeiffer and Marx[4] had occasionally observed that their animals, inoculated with the cholera vibrio, early exhibited an agglutinative power in the spleen; but this result was not met with sufficiently constantly to enable them to draw a positive conclusion. A little later, van Emden[5] studied in detail the distribution of the agglutinative property in the body of an animal inoculated with *Bacillus aërogenes*. His researches led him to the conclusion that the spleen and the lymphoid organs must be regarded as the source of the agglutinins.
[278] Shortly after the inoculation of the bacilli, an extract of the spleen was more agglutinative than the blood or any of the other organs. In rabbits from which the spleen had been removed, the same *rôle* was filled by the bone marrow and probably also by the lymphatic nodules. But this preponderance of the haematopoietic organs did not continue long, the blood soon becoming the most important seat of the agglutinative power.

The proof that this question of the origin of the agglutinins is a very delicate and difficult one is afforded by an investigation very carefully carried out by Gengou[6] on the agglutination of the attenuated anthrax bacillus (Pasteur's first vaccine) by the fluids and organs of normal and prepared guinea-pigs. This observer was never able to obtain any confirmation of the results obtained by van Emden with another micro-organism. In Gengou's guinea-pigs it was always the blood fluid which showed itself most agglutinative, the organs exhibiting merely a feeble and inconstant agglutinative power. As the

[1] *Arch. de méd. expér. et d'anat. path.*, Paris, 1896, 1re série, t. VIII, p. 759.—Bensaude, "Le phénomène de l'agglutination des microbes," Paris, 1897, p. 252.
[2] *Compt. rend. Soc. de biol.*, Paris, 1897, p. 104.
[3] *Ann. de l'Inst. Pasteur*, Paris, 1897, t. XI, p. 376.
[4] *Ztschr. f. Hyg.*, Leipzig, 1898, Bd. XXVII, S. 272.
[5] *Ztschr. f. Hyg.*, Leipzig, 1899, Bd. XXX, S. 19.
[6] *Arch. internat. de Pharmacodyn.*, Gand et Paris, 1899, vol. VI, p. 299.

extracts of leucocytes were always found to be markedly less active than the blood and the fluids of the exudations, Gengou was obliged to come to the conclusion that the agglutinins cannot be regarded as products of the cells of the animal body; this he sums up by saying that "in the increase of the agglutinative power of its blood the organism of the animal plays only a relatively passive part" (p. 337).

I think that, in spite of the facts established by Gengou, his conclusion can scarcely be regarded as final. The agglutinative property, developing in the animal body, must be attributed to some cellular influence, because we know that the prolonged sojourn of micro-organisms in the animal fluids is incapable of conferring on them this power. As Gengou's experiments did not permit him to attribute the formation of agglutinin to any formed element, it must be concluded that, although perfectly exact, they were insufficient to solve the problem. Gengou killed his animals at a stage when their blood was already pretty strongly agglutinative. At this stage the organs only possessed it to a much more feeble degree. Perhaps, if he had examined his animals at an earlier stage, when the blood possessed a much less marked agglutinative power, he might have obtained a more powerful agglutination with an extract of the organs. In my researches on the resorption of cells, I observed, on several occasions, that the abdominal fluid of guinea-pigs which had [279] received an injection of goose's blood became agglutinative before the blood serum. Later, however, the blood exhibited a greater agglutinative power than did the peritoneal fluid. If to this fact we add the results of van Emden's experiments, we shall be tempted to assign to the cells found in the peritoneal exudation and in the lymphoid organs a share in the production of the agglutinin. This question of the origin of the agglutinative power is, however, a very difficult one, and it is impossible, in the imperfect state of our knowledge, to express oneself in a more positive fashion. Fortunately, according to the whole of our data on this phenomenon, the part played by agglutination in immunity can only be very inconsiderable, and we may be allowed to consider our general problem without concerning ourselves over much about the origin of the agglutinative property.

Among the definite results obtained from the study of the agglutinins, it may be specially pointed out that these substances can in no way be identified with the fixatives. These latter were, for

long, spoken of as *preventive substances*. They are so termed in the early papers of Jules Bordet treating upon this question. The explanation of this designation is that, for a series of years, the presence of the fixatives was revealed chiefly by the preventive or protective property of the media which contained them.

To gain a clear conception of this protective property, which occupies so important a place in the study of acquired immunity, we must go back to an epoch in our science when it was sought to prove that the fluids of the body played a part in the production of immunity. Shortly after the earliest researches on the bactericidal power of the blood had been made, the idea of applying the results obtained in this direction to the production of immunity in animals by means of injections of blood occurred. The first step in this direction was taken by Richet and Héricourt[1], who succeeded in vaccinating rabbits against a variety of staphylococcus by means of defibrinated dog's blood. The dog is naturally refractory against this organism, and the blood of a normal dog exercised a certain vaccinal or protective influence on rabbits inoculated with the staphylococcus. But this action was much more marked when Richet and
[280] Héricourt employed the defibrinated blood of dogs which had previously received inoculations of the staphylococcus. Shortly after this observation, von Behring[2] made his discovery of antitoxins in the blood serum of animals immunised against tetanus and diphtheria toxins. In collaboration with Kitasato he demonstrated that the serum of these animals, when injected into normal animals, protected them against intoxication by the poisons of diphtheria and tetanus. This great discovery, which has been confirmed on all sides and extended to other poisons, gave rise to the view that a serum exerting any protective power depends solely on its property of impairing the action of the toxins. A more careful study of the phenomena which appear under the influence of the serums has, however, demonstrated the inaccuracy of this view. I was able to furnish the proof[3] that the blood serum of rabbits vaccinated against the micro-organism of the Gentilly pneumo-enteritis prevented normal rabbits from contracting a fatal infection. Nevertheless, the serum exerted no influence on the toxin of this

[1] *Compt. rend. Acad. d. Sc.*, Paris, 1888, t. CVII, p. 750.
[2] Behring u. Kitasato, *Deutsche med. Wchnschr.*, Leipzig, 1890, S. 1113.
[3] *Ann. de l'Inst. Pasteur*, Paris, 1892, t. VI, p. 299.

micro-organism; the rabbits that received the minimal lethal dose of this toxin, mixed with serum from vaccinated rabbits, died, as did the control animals, from rapid poisoning. It was evident then that this serum, which prevented infection without in any way hindering intoxication, could not be classed in the category of anti-toxic serums. We find ourselves, therefore, in the presence of a new property of the fluids of the body to which we have given the name of *protective* or *anti-infective power*. We are driven to this conclusion the more as the serum in question was neither bactericidal nor agglutinative.

This discovery was soon confirmed by R. Pfeiffer[1] for the cholera vibrio. Animals vaccinated against this organism furnished Pfeiffer with a serum which, whilst not at all antitoxic, was distinctly protective when injected into normal guinea-pigs. It protected these animals from a fatal infection by the vibrio and, when injected into the peritoneal cavity, it set up the granular transformation of the cholera vibrios,—Pfeiffer's phenomenon. Pfeiffer, for this reason, gave to the protective anti-vibrio serum the name of bactericidal serum. As the granular transformation was produced, under the [281] influence of this serum, with cholera vibrios only and never with other species of vibrio, Pfeiffer gave to the active substance in the serum the name of *specific cholera-antibody*. This substance, according to his theory, was formed in the animal body at the expense of an inactive antibody which became transformed into an active substance under the influence of the peritoneal endothelium.

The possibility of thus vaccinating susceptible animals by means of the serums of immunised animals, quite apart from any anti-toxic power, was easily confirmed and extended to several other infective diseases. Pfeiffer and Kolle[2], Funck[3], Chantemesse and Widal[4] demonstrated it in connection with the experimental disease produced in animals by the typhoid bacillus; Loeffler and Abel[5] for the *Bacillus coli*, etc. The protective or anti-infective power of the serum and other fluids of immunised animals was soon recognised as a general property.

[1] *Ztschr. f. Hyg.*, Leipzig, 1894, Bd. XVI, S. 268; 1894, Bd. XVIII, S. 1.

[2] *Ztschr. f. Hyg.*, Leipzig, 1896, Bd. XXI, S. 203; *Deutsche med. Wchnschr.*, Leipzig, 1896, SS. 185, 735.

[3] "La sérothérapie de la fièvre typhoïde," Bruxelles, 1896.

[4] *Bull. Soc. méd. d. hôp.*, Paris, 1893, 27 janvier.

[5] *Centralbl. f. Bakteriol. u. Parasitenk.*, Jena, 1896, I^te^ Abt., Bd. XIX, S. 51; *Festschr. z. 100jähr. Stiftungsfeier d. med. chir. Friedr. Wilh. Instituts*, 1895.

Pfeiffer and his collaborators, as well as many other investigators, laid special stress on the bactericidal character of these protective fluids. It was seen that the serums of immunised animals were often almost or completely incapable of killing the corresponding micro-organisms, but they were still regarded as bactericidal, because, when injected into the peritoneal cavity of normal animals, they set up the transformation of vibrios into granules, or, in the case of other bacteria, determined certain phenomena of extracellular destruction. Whilst carrying on researches in this direction, Fränkel and Sobernheim[1] discovered a fact of great importance. They found that the protective substance of the serum of animals vaccinated against the vibrios resisted heating to 70° C. When submitted to the influence of this temperature, the serum lost its bactericidal power completely, but remained quite as protective as the unheated serum, when injected into susceptible animals. This experiment, which has since been confirmed repeatedly, furnished us with a means of separating the bactericidal power from the
[282] protective power in cases where both were present in the same serum. Later, in the hands of Bordet, it proved to be of great service in connection with his researches on the concurrence of two substances in acquired immunity.

The possibility of obtaining Pfeiffer's phenomenon outside the body by "reactivating" the protective serum with peritoneal fluid or blood serum of normal unvaccinated animals has still further facilitated the study of the action of the two substances in acquired immunity. It was with the help of this method that Bordet was able to furnish so much valuable information on the subject of anti-cholera serums and, later, on that of haemolytic serums. The discovery by Ehrlich and Morgenroth[2] of the fixation by the sensitive elements of the heat-resisting (thermostabile) substance (that which resists a temperature of 65°—70° C.) constitutes a new and important acquisition to the study of acquired immunity. The discovery has been applied by Bordet to micro-organisms, and since then it has been found possible to study much more precisely the mode of action of specific protective serums.

Even before this last scientific advance had been made it was possible to determine the relations between the protective power and the agglutinative power of the fluids of animals that had acquired

[1] *Hygien. Rundschau*, Berlin, 1894, IV Jahrg., SS. 97, 145.
[2] *Berl. klin. Wchnschr.*, 1899, S. 6.

immunity. Both resist about the same temperatures; both are found in the blood plasma and pass into the fluids of exudations and transudations. But it may be affirmed with certainty, as already stated, that the two properties are quite distinct. Pfeiffer has laid great stress on the fact that highly protective serums often exhibit only a feeble agglutinative power and *vice versa*. During an investigation[1] into an epidemic of typhoid fever, he had occasion to study the serum of patients convalescent from this disease. The exact dosage of the two properties demonstrated that a slightly marked agglutinative property might be associated with a very powerful protective property. Gheorghiewsky[2] made similar observations on animals vaccinated against the *Bacillus pyocyaneus*. The serum of a goat, although more agglutinative, invariably proved to be less protective than that of a rabbit. A similar result was obtained with the serum of immunised guinea-pigs. "This [283] shows distinctly"—concludes Gheorghiewsky—"that the property possessed by serums of agglutinating the *Bacillus pyocyaneus* does not march parallel with the protective property" (p. 304). Analogous examples are sufficiently numerous to justify us in accepting the distinctiveness of the two properties of specific serums.

The protective or anti-infective substance is, therefore, not the same as the agglutinin. But are we justified in regarding it as identical with the fixative substance, or fixative (sensibilising substance, immunising or intermediary substance, or amboceptor)? From the fact that the fixative was at first rightly designated by Bordet as protective substance we should conclude in the affirmative. The question is an important one and merits close examination. The discovery of an exact method of determining the presence of fixatives has rendered it possible to ascertain whether these substances are always found in the protective fluids and also whether the presence of fixatives necessarily implies the protective power of the serums.

The first of these questions has been answered in the affirmative. All the protective serums studied from this point of view, by Bordet and Gengou, were found to be endowed with very distinct fixative properties. They also found the specific fixative in the serum of guinea-pigs immunised with the attenuated bacilli of the first vaccine of Pasteur. Now this serum is powerless to prevent the production

[1] "Typhusepidemien und Trinkwasser," Jena, 1898, S. 26.

[2] *Ann. de l'Inst. Pasteur*, Paris, 1899, t. XIII, p. 298.

of fatal infection in mice into which is simultaneously injected the bacillus of the first vaccine. Consequently a fixative fluid is not necessarily protective. This is in accordance with the fact that the micro-organisms that have absorbed the fixative may, nevertheless, retain their virulence. We have already cited the experiment of Mesnil that the bacilli of swine erysipelas, mixed with the specific serum and then deprived of this fluid, produce a fatal infection in mice. We have also drawn attention to the fact, demonstrated by Sawtchenko, that anthrax bacilli, obtained from the exudation of immunised rats, give rise to a fatal anthrax in normal guinea-pigs and rats. The experiments of Bordet and Gengou proved that there is absorption of the fixative substance by the bacilli of swine erysipelas and of anthrax when placed in contact with the specific serums of the immunised animals. In order that the protective power may manifest itself adequately, therefore, besides the fixative substance, some other factor capable of acting is also necessary.

[284] In connection with my work on immunity against the micro-organism of swine pneumo-enteritis I was able to demonstrate that the serum of vaccinated rabbits, incapable of preventing the multiplication of the specific cocco-bacillus, is also powerless to deprive it of its virulence; it is without the power of causing its agglutination or of neutralising its toxin. In short, this serum appears to exercise no direct action on the micro-organism, yet, in spite of that, it prevents its pathogenic action. With these results before me, I was led to assume a certain stimulating action of the serum on the defensive elements of the animal organism and especially on the phagocytic system. The discovery of the fixative property of serums would lead us to believe that this stimulation was entirely useless, and that the permeation of micro-organisms by the fixative was amply sufficient to bring about their destruction and removal from the animal. A living micro-organism in its normal form, endowed with full virulence and provided with its fighting weapon, the toxin, but at the same time permeated by the fixative substance, might behave in the animal in some special way. It might excite a strong positive chemiotaxis of the leucocytes and be ingested and destroyed by these cells with greater facility. *A priori*, there would be nothing to object to in this view, but certain facts are opposed to it. Thus, in the case of micro-organisms just cited, we see bacteria, permeated not only with the fixative but also with cytases, capable of producing a fatal infection. We are thus compelled to accept the theory of an

influence of protective serums not only on the micro-organisms but also on the organism of the animal into which they are introduced. As this influence manifests itself in the form of a strong phagocytosis, it is only natural that we should attribute it to the existence of a *stimulating action* of the serums of vaccinated animals on the phagocytes of the normal animals. The detailed analysis of the mechanism of the immunity acquired as the result of the injection of these serums, as we shall attempt to prove in the following chapter, in many cases confirms this view.

The important part played by the stimulation of the phagocytic reaction in acquired immunity is supported by yet another series of facts and from a different side. It has been clearly established that not only the serum of immunised animals but also that of normal man and normal animals, themselves susceptible to the pathogenic action of the micro-organisms, protects the animal organism against infection. This fact was first demonstrated in connection with researches on the vaccination of guinea-pigs against the experimental [285] peritonitis produced by the cholera vibrio.

G. Klemperer[1] was the first to observe that the blood of several individuals who had never had cholera was, nevertheless, in the case of guinea-pigs, protective against peritoneal infection by the cholera vibrio. He concluded therefrom that the individuals who had furnished this protective blood possessed immunity against cholera. Soon afterwards I[2] was able to extend analogous researches over a large number of persons and to show that the protective power of the blood is of very wide distribution in human beings. But, instead of assuming that all these individuals, whose fluids protect the guinea-pig from peritoneal infection, possess a natural immunity against cholera, I came to the conclusion that the protective power of the blood cannot be taken as a measure of the immunity of the individual from whom the blood was drawn. Here again I assumed a stimulant action of the human blood on the phagocytic reaction of the guinea-pig, looking upon it as quite natural that the blood, capable of exciting the reaction in an alien animal, might remain inactive in the body of the animal which furnished it.

R. Pfeiffer[3] has given much attention to the protective action

[1] *Berl. klin. Wchnschr.*, 1892, S. 970.
[2] *Ann. de l'Inst. Pasteur*, Paris, 1893, t. VII, p. 411.
[3] *Ztschr. f. Hyg.*, Leipzig, 1894, Bd. XVI, S. 268.

of serums; he has laid special stress on the essential difference between the influence of normal serums and of those obtained from animals that have acquired immunity. Whilst, in order to obtain a protective effect with the normal blood or serum of man and animals, it is necessary to inject a considerable quantity (from 0·5 c.c. upwards), the specific serum, i.e. serum obtained from persons recovered from cholera or from animals vaccinated against the cholera vibrio, is active in a very minute dose. Sometimes the cholera peritonitis of the guinea-pig is prevented by a fraction of a milligramme of such serum[1]. Based on these facts, Pfeiffer has expressed the view that the normal serum acts by stimulating the natural powers of defence of the animal, whilst the specific serum exercises its influence in virtue of the property of causing the formation of a special secretion which acts only against the micro-organism which served for the production of the immunity. Pfeiffer and his collaborators have demonstrated that normal serums are protective, not only against the cholera vibrio, but also against several other micro-organisms, e.g. the typhoid bacillus. One of his
[286] pupils, Voges[2], believed that, in certain infections, the protective power of normal blood might be greatly exaggerated, and that, in these cases, the limit between the activity of normal and of specific serums might be almost completely effaced. He affirmed, especially, that very small doses (0·1 c.c.) of blood serum from a normal guinea-pig was quite sufficient to prevent, in other guinea-pigs, a fatal infection by the micro-organism of hog cholera and its allies. As this fact might be of general application I asked M. Saltykoff[3], who was working in my laboratory, to verify the statements of Voges. Several series of experiments demonstrated the incorrectness of the contention. The small doses of normal serum of guinea-pigs, indicated by Voges, were found to be absolutely incapable of protecting against the virus used by him in his experiments.

The fact that normal serums, injected in sufficiently large doses, exhibited an undoubted protective property, affords additional proof that this property cannot be identified with the fixative power. The latter was present in serums which were not protective; here, then, we have the inverse phenomenon and we see

[1] See Lazarus, *Berl. klin. Wchnschr.*, 1892, S. 1072.
[2] *Ztschr. f. Hyg.*, Leipzig, 1896, Bd. XXIII, S. 149.
[3] *Ann. de l'Inst. Pasteur*, Paris, 1902, t. XVI, p. 94.

normal serums exercise their protective action although they contain no fixative. This follows from Bordet and Gengou's experiments already described, according to which the cytases, placed in contact with micro-organisms in normal serums, remain free, simply because of the absence of fixatives.

We are led, then, from these demonstrations to recognise the presence of stimulins not only in specific serums, but also in normal serums. Between the two there is this difference that, when applied with the normal fluids, the stimulins alone act, whilst when injected with the serum of the animal enjoying acquired immunity the action of the stimulins is facilitated and reinforced by the fixatives or sometimes, perhaps, by the agglutinins.

The stimulating influence of certain normal serums may be so considerable that it may prevent infection by the micro-organism, injected at the same time in a dose many times more than lethal. Wassermann[1] protected guinea-pigs by injecting into the peritoneal [287] cavity a quantity as great as 40 times the lethal dose of typhoid bacilli, by introducing at the same time and at the same place 3 c.c. of normal rabbit's serum, heated to 60° C. Besredka[2], who confirmed this observation, has analysed its special mechanism. He showed that the serum exercises a very marked stimulating influence on the guinea-pig's leucocytes, which then exhibit a truly extraordinary phagocytic activity. They are seen to act in the peritoneal fluid, but they are much more active in the region of the omentum, where the leucocytes gorge themselves with micro-organisms, devouring them by dozens. The stimulating action of the heated rabbit's serum is exercised in a similar fashion if, instead of micro-organisms, grains of carmine be injected. Very shortly after the commencement of the experiment very little carmine is found outside the cells ; it is all either ingested by individual leucocytes, if the grains are small, or surrounded by numerous leucocytes when the grains are massed together ; this phagocytosis is most developed in the region of the omentum, exactly as in the case of typhoid bacilli.

These facts, which so clearly demonstrate the stimulating action of the normal rabbit's serum, prove in another way that the stimulin resists heating to 60° C., and that, in this respect, it resembles the agglutinins and fixatives. This may afford us an indication as to the nature of the stimulating substance. The possibility of obtaining an

[1] *Deutsche med. Wchnschr.*, Leipzig, 1901, S. 4.
[2] *Ann. de l'Inst. Pasteur*, Paris, 1901, t. xv, p. 209.

antistimulin gives us another valuable indication. Wassermann, in the work we have just cited, showed that the serum of a rabbit, previously treated with guinea-pig's serum and injected under the same conditions as in the experiment with normal rabbit's serum, has completely lost its protective power. The typhoid bacilli multiply freely in the peritoneal cavity and the organism of the guinea-pig is incapable of opposing a sufficient resistance. Wassermann thinks that, in this case, the disease becomes grave because of the anticytase found in the serum of rabbits treated with guinea-pig's blood. There is no doubt that this serum is really anticytasic. But as the free cytases found in the peritoneal cavity of a guinea-pig inoculated at the moment of phagolysis, become inactive under the influence of the anticytase and play merely a minor part, it is impossible to ac-
[288] cept the German investigator's interpretation. Indeed, Besredka has proved that, in this case, it is the antiphagocytic or antistimulant action of the rabbit's serum which brings about the fatal issue in the case of the typhoid inoculation.

We have laid stress on the point that an animal, whose serum is protective when introduced into another animal, may itself not be refractory against the specific micro-organism. As regards the serum of normal unvaccinated animals this has been so fully demonstrated that nowadays no one doubts it. The question is more complicated in the case of animals that have acquired immunity. As in the great majority of cases the serum of these animals is found to be endowed with a very great protective power, it has been accepted as proved that the animal which furnishes it must itself possess great immunity. The degree of protective power has even been taken as the measure of the acquired immunity. Thus, the numerous attempts to vaccinate the human subject against typhoid fever, undertaken in consequence of the researches of Pfeiffer and Kolle[1], were based on the fact that in these cases the serum of vaccinated individuals acquires a great protective power. It was argued that if this power is present it can only be due to the acquired immunity of the individuals who furnish such a serum. Undoubtedly the protective property of the fluids and the resistance are often equal ; but it is none the less true that there are cases where, in spite of this property being markedly developed, the animal that furnishes the protective serum is susceptible to the action of the micro-organism and may even succumb to infection therewith.

[1] *Ztschr. f. Hyg.*, Leipzig, 1896, Bd. xxi, S. 203.

As the hypothesis just mentioned is of importance from a general point of view it must be supported by adequate proof. It was during the course of the vaccination of rabbits against the micro-organism of the pneumo-enteritis epidemic at Gentilly that I was first able[1] to assure myself of its accuracy. I noticed that some of these rabbits, although vaccinated, ultimately succumbed to pyaemia, set up solely by this micro-organism. They were consequently not refractory against the disease, and yet their blood serum, when injected into normal rabbits along with an absolutely fatal dose of micro-organisms, was found to be highly protective. This observation drove me to the conclusion that the protective power is not a function of immunity and cannot be received as a measure of this immunity. Analogous facts have since been demonstrated in certain other cases. Thus, Pfeiffer[2] on several occasions has [289] found that guinea-pigs, highly immunised against the cholera vibrio, have succumbed after the injection of a moderate quantity of these organisms. "On post-mortem examination of these cases living vibrios were found in the peritoneal cavity, sometimes in considerable numbers; and yet minimal doses of the heart blood given to normal guinea-pigs caused in these animals a very marked breaking down of the vibrios." Alongside these facts may be placed others, described in the preceding chapter, of well-immunised animals dying from infection, after they had been weakened by opium, cold, or other lowering agent. It is clearly seen, then, that for the manifestation of acquired immunity it is necessary that the reaction of the living cell elements should take place without let or hindrance. When this reaction fails, the possession of even great protective power is insufficient to prevent the immunised animal from contracting a fatal infection.

If, in acquired immunity against micro-organisms, it is really the cell defence which plays the most important part, we can readily imagine cases where it by itself can confer immunity without calling in the co-operation of the protective power of the fluids. When in this connection we study the resistance of an animal against various pathogenic organisms, we note, first of all, the very great variability that exists in the production of the acquired humoral properties. In certain cases, as in vaccination against vibrios or typhoid bacilli, the serum very readily becomes not only protective, but agglutinative and fixative. In other cases these properties develop with difficulty

[1] *Ann. de l'Inst. Pasteur*, Paris, 1892, t. VI, p. 300.
[2] *Ztschr. f. Hyg.*, Leipzig, 1895, Bd. XIX, S. 82.

and are only manifested after a long period of vaccination. Such is the case with anthrax. After the discovery of protective serums, numerous attempts were made to obtain a serum protective against the anthrax bacillus. Several observers failed in their attempts, others were more fortunate. Sclavo[1] and Marchoux[2] were the first to succeed in obtaining a protective serum from animals hyper-immunised against anthrax. They were able to show that the serum of sheep, treated first with vaccines and then repeatedly with anthrax virus, would protect rabbits against a fatal dose
[290] of the bacillus. Marchoux even obtained, with hyperimmunised rabbits, a serum which prevented normal rabbits from contracting fatal anthrax. Sobernheim[3] was less fortunate in his first experiments. He satisfied himself that the blood serum of cattle that had recovered spontaneously from anthrax or that had been vaccinated according to Pasteur's method, was absolutely unable to protect small animals against the anthrax bacillus, and his hyper-vaccinated rabbits furnished serums of doubtful activity. It was only later that he succeeded[4] in obtaining better results; especially when he used sheep. Even then he found that in the production of the anti-infective property the individuality of the immunised animals had a dominant influence. Thus, in two sheep, treated in exactly the same way, the serum of one was found to be incapable of protecting a rabbit, whilst that of the other exhibited an undoubted, although feeble, protective power.

But what is of greater interest to us, from our point of view, is that guinea-pigs which have been vaccinated against anthrax and which enjoy a considerable immunity against this disease, exhibit no protective power. In a letter from Behring I learnt that this fact had for the first time been demonstrated by Wernicke in experiments carried out in the Hygienic Institute at Marburg. After repeated and painstaking attempts this observer succeeded in vaccinating guinea-pigs against enormous doses of virulent anthrax bacilli. The serum from the animals so immunised was, however, quite incapable of protecting normal guinea-pigs against a fatal infection. This result was the more extraordinary since Wernicke's pigeons, likewise vac-

[1] [*Centralbl. f. Bakteriol. u. Parasitenk.*, Jena, 1895, Bd. XVIII, S. 744]; *Riv. d'Ig. e San. Pubbl.*, Torino, 1896, t. VII, nos. 18—19; *ibid.* 1901, t. XII, p. 212.

[2] *Ann. de l'Inst. Pasteur*, Paris, 1895, t. IX, p. 785.

[3] *Ztschr. f. Hyg.*, Leipzig, 1897, Bd. XXV, S. 301.

[4] *Ztschr. f. Hyg.*, Leipzig, 1899, Bd. XXXI, S. 89.

cinated against anthrax, gave a serum whose protective power was quite distinct. Realising the great importance of these facts I asked M. de Nittis[1] to repeat these experiments in my laboratory. The vaccination of pigeons is an easy matter, but that of guinea-pigs presents great difficulties. He succeeded, nevertheless, in vaccinating some of these rodents very highly, and this enabled him to compare the protective power of the blood serum in the two species. That of the vaccinated pigeon was found to be endowed with this power and protected guinea-pigs and mice against virulent anthrax. The serum of the immunised guinea-pigs, on the contrary, exhibited no pro- [291]
tective property, just as in Wernicke's experiments. The guinea-pigs and mice, into which this serum was injected at the same time as the anthrax bacilli, died even when attenuated anthrax was used. We have, then, in this case, an example of acquired immunity, independent of any protective power of the fluids of the body.

In the course of their researches on the bacillus isolated by R. Pfeiffer from persons attacked by influenza, Delius and Kolle[2] tried to vaccinate susceptible animals (guinea-pigs) against this minute organism and to immunise animals naturally refractory (dog, sheep, goat) against fairly large doses of cultures. They succeeded in vaccinating guinea-pigs against ten times the lethal dose, but never obtained any protective serum. Nor did the other animals that were treated furnish a protective serum. "From the whole of our experiments carried on for several years"—conclude Delius and Kolle—"it is quite evident that we were unable to produce any appreciable change in the blood by the use of those methods which have produced specific immunising serums against other bacteria such as the bacilli of diphtheria, cholera, typhoid fever, and 'blue pus'" (p. 345). Slatineano undertook a detailed study of Pfeiffer's bacillus in my laboratory, but he found it impossible to demonstrate any unquestionable protective effect exerted by the blood serum of vaccinated guinea-pigs upon normal guinea-pigs inoculated with a fatal dose of this organism. We are not justified, therefore, in classing this bacillus with the anthrax bacillus; we may, however, cite it as an argument illustrating the difficulty that is met with, in certain examples of acquired immunity, of discovering the protective power, when feeble and masked.

The inoculation with micro-organisms of animal nature causes the

[1] *Ann. de l'Inst. Pasteur*, Paris, 1901, t. xv, p. 769.
[2] *Ztschr. f. Hyg.*, Leipzig, 1897, Bd. xxiv S. 327.

development of acquired immunity, but in this case the properties of the fluids of the body are but little in evidence or they may be even *nil*. Let us return to the example of the *Trypanosoma* of the rat which excites in vaccinated animals a protective and weakly agglutinative power of the serum. This fluid, however, is usually found to be incapable even of rendering the flagellated parasites motionless.

The question of immunity against malaria has been much dis-
[292] cussed. It is well known that a first attack of this disease, so far from conferring any immunity of the slightest durability, leaves a certain predisposition to another attack. In spite of this the study of malaria in various countries and in individuals belonging to different races has demonstrated that there does indeed exist a certain degree of acquired immunity against this disease. During recent years Koch[1] has paid special attention to this subject and has furnished us with very valuable data, based especially on a comparative study of the blood of children and adults. The frequency of Laveran's parasite in the former and its rarity in the latter, have led him to the conclusion that infantile malaria sets up an immunity which persists in the adult. Moreover, it has been established that in malarial countries the indigenous inhabitants exhibit an attenuated form of the disease, unaccompanied by acute attacks, but with phenomena that are chronic and very slow in development.

In spite of the existence of a certain degree of acquired immunity against malaria, all attempts to demonstrate any protective action of the serum have been fruitless. Celli[2], indeed, injected, as a preventive, the blood serum of individuals who had recovered from malaria or of others who were bled during the period of defervescence following an acute crisis of this disease, but in every instance these injections were found to be useless in preventing an attack of malaria.

We can readily understand that in a disease which is exclusively human, such as malaria, it has not been possible to perform a sufficient number of experiments to decide the question of the protective property of the blood. In this respect we shall have

[1] *Deutsche med. Wchnschr.*, Leipzig, 1900, S. 781.

[2] "La Malaria secondo le nuove recherche," Roma, 1899, p. 86 [translated into English by Eyre from the 2nd Italian edition under the title "Malaria according to the new researches," London, 1900]. "Die Malaria" [German translation of same] in Behring's "Beiträge z. exper. Therapie," 1900, Bd. 1, Hft. 3.

greater chance of obtaining satisfactory data if we direct our attention to some analogous disease attacking one of the lower animals. Such a disease we have in Texas fever, occurring in the Bovidae, as the result of the action of an animal parasite, *Piroplasma bigeminum*, which invades the red blood corpuscles much as Laveran's parasite invades those of the human subject.

As mentioned in the preceding chapter, Smith and Kilborne and Koch have demonstrated that the Bovidae may acquire a real immunity against Texas fever. Nicolle and Adil Bey[1] at [293] Constantinople found indigenous races that exhibited a remarkable immunity against the *Piroplasma*. Having demonstrated this fact the idea occurred to them to inoculate these refractory cattle with very large quantities of virulent blood and to make use of the serum from animals so treated for the prevention of infection in susceptible races of Bovidae. This experiment gave negative results. Lignières[2] elaborated a special method of vaccinating susceptible Bovidae and was successful in obtaining very encouraging results. A commission of veterinary surgeons from Alfort[3] appointed to verify these observations came to the conclusion that "the vaccination as carried out by Lignières was absolutely effective."

Lignières also carried out researches on the protective power of the blood serum of his immunised cattle. In a communication to the International Congress of Medicine, held in Paris in 1900, he stated that the injection of several hundred cubic centimetres of this fluid did not protect normal animals against infection. We must conclude, therefore, that, here also, we have another example of acquired immunity unaccompanied by the presence of any protective property of the blood fluid.

These results have received confirmation from a most authoritative source. Nocard has kindly communicated to me the fact that he has tried in vain to confer immunity on normal dogs into which he has injected blood serum coming from dogs that had recovered from the disease produced by a haematozoon closely allied to that of Texas fever or serum from sheep immunised with blood from the affected dogs.

Looking at the data we have just summarised as a whole, we are

[1] *Ann. de l'Inst. Pasteur*, Paris, 1899, t. XIII, p. 343.

[2] "La 'Tristeza' ou Malaria bovine dans la République Argentine," Buenos Ayres, 1900, p. 142.

[3] *Bull. Soc. centr. de méd. vétérin.*, Paris, 1900, séances des 12 et 26 juillet.

compelled to recognise that, on the one hand, the protective power of the body fluids may coincide with a susceptibility to the corresponding micro-organism, and that, on the other, real acquired immunity may exist without any manifestation of this humoral property, especially as, even in immunised animals, the acquired immunity often persists longer than does this property. It must be accepted then, that, in this immunity, there exists something other than the powers of the fluids of the body, that is to say, the factor which plays the predominant part is to be sought for in the
[294] cellular elements. We need only recall the many facts collected in the preceding chapter to be convinced that in acquired immunity phagocytosis is the most constant and most general phenomenon. We find it in cases where the humoral properties are the most marked, as well as in those in which they are only slightly developed or are entirely absent. We need not again discuss Pfeiffer's phenomenon analysed in the preceding chapter. It is sufficient to mention that this example of the extracellular destruction of micro-organisms only occurs under limited and special conditions. It is observed only in cases where the injection is made into a situation rich in leucocytes which undergo phagolysis as a result of the sudden change brought about in their conditions of existence. Further, this phenomenon is observed only in connection with micro-organisms that are slightly resistant to the influence of the microcytases. In those cases in which we meet with Pfeiffer's phenomenon, we also meet with a widely extended phagocytic reaction.

This reaction is most pronounced where the properties of the body fluids are only slightly developed or are absent. The study of acquired immunity against anthrax provides us with a very convincing proof of this. We have already cited the example of vaccinated rabbits and rats in which phagolysis is incomparably greater than in the susceptible control animals which contract a fatal anthrax. This rule is general. It is confirmed in the vaccinated sheep and guinea-pig. The absence, or feeble development, of the protective power of the blood or of the other humoral properties in no way, then, prevents the considerable change which is set up in the phagocytes of animals that have acquired immunity against anthrax. The negative chemiotaxis of the leucocytes, so marked in susceptible animals, is modified into positive chemiotaxis as the result of vaccination. This fact, one of fundamental importance, was first demonstrated for the immunity against anthrax, later being ex-

tended to other micro-organisms. Massart[1] studied the general subject and collected a series of data which led him to say that "vaccination effects an education of the leucocytes; these latter become so adapted that they can approach the virulent micro-organisms." The best method of forming an estimate of the change which the leucocytes undergo is by injecting subcutaneously very [295] virulent micro-organisms capable of setting up a generalised infection. The anthrax bacillus, Gamaleia's vibrio, the streptococci and the cocco-bacilli of swine and fowl cholera are very suitable for such study. These micro-organisms, when inoculated subcutaneously into susceptible animals, set up a very slight local reaction or none at all, in the form of an exudation of transparent fluid almost entirely without leucocytes. The micro-organisms grow freely in these exudations and soon invade the animal. In vaccinated animals the local reaction is more marked and the exudation, very rich in leucocytes, is poor in fluid; the micro-organisms remain free for a very short time, being soon ingested by the leucocytes. Their destruction, inside these cells, takes a longer or shorter time according to circumstances; but in the end it is always complete.

The difference as regards phagocytic reaction between susceptible and vaccinated animals, such as I have just described, has been generally recognised by many observers. A few opponents are still found, however, who consider that they are justified in affirming that the negative chemiotaxis of the susceptible animal does not exist and that, consequently, vaccination can in no way change it into positive chemiotaxis. Werigo made himself the spokesman of this view, which he has maintained in several papers[2]. Instead, however, of introducing the virulent micro-organisms into the subcutaneous tissue of susceptible animals he injected them directly into the veins. Using cultures of the anthrax bacillus and of the cocco-bacillus of fowl cholera he injects these into the venous system of normal rabbits. The animals soon die from general infection. If, however, these animals are killed shortly after inoculation, it is found on examination of sections that many of the micro-organisms have been ingested by the leucocytes. Werigo concludes from these facts that in the higher animals the chemiotaxis is always positive; but that it ends in the destruction of the micro-organisms in the vaccin-

[1] *Ann. de l'Inst. Pasteur*, Paris, 1892, t. VI, p. 321.

[2] *Ann. de l'Inst. Pasteur*, Paris, 1894, t. VIII, p. 1; *Arch. de méd. expér.*, Paris, 1898, t. X, p. 725; *Arch. russes de Path.* &c., St Pétersb., 1898.

ated animals, never bringing about this result in susceptible animals. Taking all the data on this question into consideration, it is easy to convince oneself that this view cannot be accepted as correct, for not
[296] only the definite phenomena observed below the skin but also the no less demonstrative process appearing in the peritoneal cavity prove most clearly the existence of this negative chemiotaxis of the leucocytes. I need only recall Bordet's experiment on the fate of streptococci and *Proteus vulgaris* when injected together into the peritoneal cavity of guinea-pigs. Whilst the *Proteus* bacilli at the end of a very short time are all ingested by the leucocytes, the streptococci remain free in the peritoneal fluid up to the death of the animal. The leucocytes which exhibit a positive chemiotaxis as regards the former, manifest a negative chemiotaxis as regards the streptococci.

In spite of the great force of these arguments, the discovery of a means of reconciling the results obtained from the inoculation of micro-organisms subcutaneously or into the peritoneal cavity, with those observed after they had been injected into the blood vessels would be of great interest, and Zilberberg and Zeliony[1] have undertaken a series of experiments with this object. Following Werigo they made use of the cocco-bacilli of fowl cholera, and found, in accordance with his observations, that the intravenous injection of these organisms, obtained from cultures in nutrient media, causes a very marked phagocytosis of the cocco-bacilli. When, however, they injected into the veins of rabbits cocco-bacilli that had been grown in the peritoneal fluid of other rabbits, they found the micro-organisms free in the blood plasma and observed only a very restricted phagocytosis in the microphages of the liver. It follows from these experiments that the ingestion of the cocco-bacilli, in Werigo's experiments, was dependent on the presence of a large number of attenuated micro-organisms which were present in the cultures that he employed for his injections. Alongside these organisms, slightly or not virulent, were others, endowed with their normal pathogenic activity and quite numerous enough to set up a fatal infection. When Zilberberg and Zeliony replaced cultures on agar by the peritoneal exudation which contained virulent cocco-bacilli almost exclusively, the phagocytosis in rabbits, injected into the veins, was found to be almost suppressed. With the object of establishing whether the absence of the phagocytic reaction, in this case, really depended on negative

[1] *Ann. de l'Inst. Pasteur*, Paris, 1901, t. xv, p. 615.

chemiotaxis on the part of the leucocytes, the above cited observers performed the following experiment. They injected into the vein of [297]
a rabbit, already affected with a generalised infection by the cocco-bacillus of fowl cholera, an innocuous culture of a saprophytic staphylococcus. Post-mortem examination showed that these cocci were almost entirely ingested by the same phagocytes which refused so energetically to seize the cocco-bacilli. This experiment, analogous to that of Bordet on streptococcus and *Proteus*, compels us to reject Werigo's conclusions as to the absence of negative chemiotaxis in the phagocytes of the higher animals. I ought to add that the work of Zilberberg and Zeliony was in part executed in my laboratory so that I was able to convince myself by ocular demonstration of the complete accuracy of their statements.

Independently of these observers and even before their work appeared, Th. Tchistovitch[1] published an interesting study on the same question. He injected very virulent streptococci into the ear vein of rabbits. These micro-organisms set up a generalised and fatal infection in which phagocytosis was completely absent or nearly so. Here again was manifested a negative chemiotaxis of the phagocytes, which, henceforth, could no longer be questioned.

In certain infective diseases terminating fatally a very marked phagocytosis is observed even in susceptible animals. The most typical example of this is furnished by swine erysipelas and mouse septicaemia. We know from the researches of Koch[2], followed by those of Loeffler[3], Schütz[4] and others, that in animals which have died from these two diseases the leucocytes are gorged with small specific bacilli. A method of vaccinating animals against the micro-organism of swine erysipelas was worked out by Pasteur and Thuillier[5] and was afterwards studied by many observers. Thanks to this method it has been possible to demonstrate the phenomena which may be observed in vaccinated animals (especially rabbits). Here also a phagocytosis takes place, even more rapid and more complete than in susceptible animals. What is more important, the intra-cellular digestion of the ingested bacilli is followed by the total [298]

[1] *Ann. de l'Inst. Pasteur*, Paris, 1900, t. XIV, p. 802.

[2] "Untersuchungen über die Aetiologie der Wundinfectionskrankheiten," Leipzig, 1878. [Translated into English in the New Sydenham Society's Series, London, 1880, Vol. LXXXVIII, under the title "On Traumatic Infective Diseases."]

[3] *Arb. a. d. K. Gsndhtsamt.*, Berlin, 1885, Bd. I, S. 46.

[4] *Arb. a. d. K. Gsndhtsamt.*, Berlin, 1885, Bd. I, S. 57.

[5] *Compt. rend. Acad. d. sc.*, Paris, 1883, t. XCVII, p. 1163.

destruction of the micro-organisms in the vaccinated animals, though in the normal animals this digestion is very imperfect.

The acquisition of immunity against micro-organisms is, therefore, due not only to the change from negative to positive chemiotaxis, but also to the perfecting of the phagocytic and digestive powers of the leucocytes—a general superactivity and adaptation of the phagocytic reaction of the immunised animal is produced. This conclusion, based upon a large number of well-established facts and in complete harmony with the whole of the data at our disposal concerning acquired immunity, has been attacked by Denys and Leclef[1] in their work on the streptococcus. They base their opposition upon experiments made *in vitro* on the action of serums and leucocytes on this micro-organism. They have compared the bactericidal power of mixtures of the serums of normal and of vaccinated rabbits with leucocytes isolated from exudations from these two groups of animals. The leucocytes, whether derived from normal or from vaccinated rabbits, when mixed with normal serum were equally incapable of ingesting and destroying the streptococci. When mixed with blood serum from vaccinated rabbits, however, the two kinds of leucocytes exhibited a very marked phagocytic reaction. Denys and Leclef conclude from this that phagocytosis, although an important factor in immunity, plays merely a secondary part and is dependent on the humoral properties. The experiments and views of these two observers have been generally received by the partisans of the bactericidal theory of the body fluids as an actual proof of this theory. We cannot agree. Researches extending over a long period have shown us that the study of phagocytosis *in vitro* can give only a very inexact and imperfect idea of the course of the phenomena in the living animal. Usually the leucocytes taken from the exudations, although amoeboid, no longer fulfil their phagocytic functions at a time when in the animal they would ingest micro-organisms with the greatest rapidity. As a general rule, existence outside the living body weakens them very considerably. But in some cases, rare it is true, the leucocytes although inactive in the animal
[299] exhibit intense phagocytosis when introduced into a hanging drop of fluid from an exudation or even of urine. In any case it is very hazardous to infer from phenomena which appear under these artificial conditions what takes place in the living animal. The value of the experiments of Denys and Leclef is still further marred by

[1] *La Cellule*, Lierre et Louvain, 1895, t. XI, p. 177.

the fact that they mixed the leucocytes with blood serum. They appear to have lost sight of the fact that this fluid is far from corresponding to that which bathes the leucocytes in the living animal. The serums contain leucotoxin in greater or less quantity and it is not to be wondered at that the leucocytes when mixed with normal rabbit's serum should perish very rapidly. Further, the serum of vaccinated rabbits is agglutinative (this fact, however, was not sufficiently elucidated in 1894 when the researches of Denys and Leclef were made) and the clumping of streptococci might simulate their destruction. In a word, the experiments of these observers have been carried out under such conditions that it is impossible to base upon them a refutation of data obtained in the living animal. Moreover, in the description of the phenomena which appear in the subcutaneous tissue of rabbits inoculated with the streptococcus, Denys and Leclef provide us with arguments against their own view.

These observers introduce the same quantity of streptococci below the skin of the ear of normal and of vaccinated rabbits. In the first there is soon produced a very marked oedema of the ear, in which may be seen a number of streptococci and of leucocytes that have not ingested any micro-organisms. In the second the oedema does not develop, but at the seat of invasion a number of leucocytes come up and these soon ingest the streptococci. As we see, the phenomena manifest themselves here just as they do with the anthrax bacillus and many other micro-organisms when under analogous conditions. Denys and Leclef, indeed, recognise that, below the skin of the ear of vaccinated rabbits, the small quantity of exudation fluid is not sufficient to enable us to accept it as capable of exerting any considerable influence as regards humoral properties. Nevertheless, they think that the "serum" of this fluid may exercise a certain action, but they furnish no proof of this, and seem to ignore the fact that the plasma of the subcutaneous exudation is far from being identical with blood serum obtained outside the animal. At present it is well known that this latter fluid contains cytases which are [300] absent from the plasmas. Now, the feeble bactericidal action, if this really exists as regards the streptococcus, must be attributed to the microcytase which has escaped from the leucocytes at the time of the preparation of the serum.

To sum up, the example studied by Denys and Leclef clearly comes under the general law of phagocytic reaction in acquired

immunity against micro-organisms. It is impossible to deny that the superactivity of the phagocytes which is always found in this immunity, although readily observed, cannot be demonstrated in a rigorous fashion outside the fluids which bathe the cells. There are, however, very important analogies which may be invoked in favour of this thesis. We have already cited in our fifth chapter Delezenne's experiments on the digestion of gelatine by the leucocytes of the dog, which show in the most demonstrative fashion that these cells accustom themselves to bring about this digestion more and more quickly and this quite independently of any humoral influence.

For some time past there has been no doubt as to the fundamental fact that the phagocytes in immunised animals seize and destroy living micro-organisms. Several attempts have been made to show that such destruction of these bacteria takes place solely by the body fluids, and that the phagocytes intervene only as "scavengers" to carry off the dead bodies of the micro-organisms. The numerous observations, described in the preceding chapter, absolve us from again entering into a discussion of this question. Moreover, the majority of these opponents now recognise that micro-organisms are ingested in a living state by the phagocytes of immunised animals. Some, however, have expressed the opinion that these living micro-organisms, before becoming the prey of the phagocytes, must undergo some preliminary attenuation of virulence through the action of the body fluids. Hence the theory of the attenuating power of the fluids of the body, maintained especially by Bouchard and his pupils. During the course of our exposition of the facts concerning acquired immunity, we have several times had occasion to speak of the virulence of micro-organisms in the immunised animal. Here, therefore, we may confine ourselves to a brief summary of the observations collected on this point.

Having observed that the anthrax bacillus, when developed in the blood of immunised sheep, was incapable of giving fatal anthrax to
[301] rabbits, I expressed[1] the opinion that under these conditions its virulence had become attenuated. Later, analogous changes were shown by Charrin[2] in the *Bacillus pyocyaneus* when cultivated in the serum of immunised animals. Bouchard[3], generalising on these data, arrived at the following theory of vaccination. "The inoculation of

[1] *Ann. de l'Inst. Pasteur*, Paris, 1887, t. I, p. 42.
[2] *Compt. rend. Soc. de biol.*, Paris, 1889–1891.
[3] "Essai d'une théorie de l'infection." Berlin, 1890.

a strong virus into a vaccinated animal is equivalent to the inoculation of an attenuated virus. The attenuation, however, instead of being done beforehand in the laboratory, is brought about in the tissues of the vaccinated animal" (p. 18). Charrin and Roger[1] upheld this view, and the latter offered several new arguments in support of it. He observed that animals inoculated with pneumococci and streptococci grown in the blood serum of vaccinated animals, contracted a transient and benign disease merely, whilst the control animals, inoculated with the same micro-organisms, cultivated in normal serum, always died from generalised infection.

The discovery of the protective property of serums has thrown a new light upon these experiments. We must now ask ourselves: Does the innocuousness of micro-organisms depend not on the attenuation of the virus, but rather on the protective action of the serum itself? When, in the course of my researches on the Gentilly cocco-bacillus, I found that this organism, cultivated in the serum of vaccinated rabbits, became much less pathogenic than when it was grown in the serum of normal rabbits, I set myself to answer this question. Simple filtration through paper was sufficient to rid the organism of the serum in which it had grown. The inoculation of cocco-bacilli thus treated proved at once that their virulence was in no degree modified, and that it was the intervention of the serum that prevented the micro-organism from setting up the rapidly fatal disease. Issaeff[2], who, in my laboratory, carried out the investigation, was able to extend this to the pneumococcus. He obtained agglutinated cultures in the serum of vaccinated rabbits, and he compared their activity by injecting them (1) with, and (2) without their culture [302] medium. The difference was very marked. In the first case the infection produced was much slower in its course than in the second. The virulence of the washed pneumococci was found to be the same whether they came from a culture in normal serum or from one in immunised serum. Sanarelli[3] obtained the same result with Gamaleia's vibrio. The vibrios when grown in the serum of vaccinated guinea-pigs proved to be very virulent so soon as they were freed from the fluid in which they were grown. Later, similar demon-

[1] Charrin, *Compt. rend. Soc. de biol.*, Paris, 1890, pp. 203, 332; Roger, *ibid.*, 1890, p. 573, and *Rev. gén. d. sc. pures et appliq.*, Paris, 1891, p. 410.

[2] *Ann. de l'Inst. Pasteur*, Paris, 1893, t. VII, p. 273.

[3] *Ann. de l'Inst. Pasteur*, Paris, 1893, t. VII, p. 231.

strations were given by Bordet[1] and Mesnil[2] with respect to streptococci and to the bacilli of swine erysipelas. We must, then, conclude that we have here to do with a general law. Some experiments made by de Nittis[3] might seem to indicate an exception to such a law. He observed that anthrax bacilli when grown in the serum of vaccinated pigeons lost a part of their virulence. It must not be forgotten, however, that he grew his cultures under special conditions; the bacillus was grown for several days at 42° C., this in itself being quite sufficient to bring about a certain attenuation of virulence.

The theory of the attenuating action of the body fluids, based on the attenuation of the virus in the serum of vaccinated animals, can no longer be maintained, as it is a well-established fact that the serum, obtained outside the body, is a fluid differing in character and properties from the plasma of the living animal. We have seen up to what point this demonstration has shaken the theory of the bactericidal action of the body fluids.

It cannot be doubted that a micro-organism may undergo a certain weakening in virulence, as well as in certain other functions, in the body of the animal that has acquired immunity. But the question must be put: Is this effect obtained as the result of humoral or of cellular action? As a general rule, exudations obtained from vaccinated animals, and containing living micro-organisms, are found to be virulent when inoculated directly into susceptible animals. This fact was established by Pasteur[4] when he first carried out his researches on acquired immunity against fowl cholera. He showed that the exudations of vaccinated fowls set up a fatal disease in
[303] normal fowls, without there being the least evidence of any attenuation of the micro-organism. The same applies to the Gentilly cocco-bacillus and to the anthrax bacillus in a very great majority of examples. De Nittis observed that the exudations of immunised pigeons produced a fatal infection in the guinea-pig and in the mouse. In the immunised guinea-pig, on the other hand, he found that the exudations soon became innocuous for these animals. This alteration, however, must be attributed not to the body fluids (which exhibit no protective or attenuating power) but to the action of the cells.

[1] *Ann. de l'Inst. Pasteur*, Paris, 1897, t. XI, p. 177.
[2] *Ann. de l'Inst. Pasteur*, Paris, 1898, t. XII, p. 481.
[3] *Ann. de l'Inst. Pasteur*, Paris, 1901, t. XV, p. 769.
[4] *Compt. rend. Acad. d. sc.*, Paris, 1880, t. XC, p. 1033.

With the object of gaining some idea of the changes that the micro-organisms undergo in the immunised animal, Vallée[1] carried out a series of experiments on rabbits vaccinated against the bacillus of swine erysipelas. He enclosed these bacilli in sacs of collodion which he introduced into the peritoneal cavity of susceptible rabbits and of others that were hyperimmunised. The bacillus developed well in both cases. It gave homogeneous non-agglutinated cultures in the sacs placed in normal animals, whilst in the sacs introduced into the peritoneal cavity of hyperimmunised rabbits the bacilli grew into agglutinated filaments. This proves that the wall of the sacs permitted of the passage of the active substances elaborated in the immunised animal. Different from the point of view of agglutination, the cultures likewise exhibited a considerable difference in their pathogenic activity. The cultures developed in the sacs in hyperimmunised rabbits were found to be much more virulent than those grown in the sacs in control animals. This augmentation of virulence depends, probably, on the influence of the active substances which pass through the walls of the sacs. In any case, this experiment affords further confirmation of the impossibility of maintaining the theory of the attenuation of micro-organisms by the fluids of an animal enjoying acquired immunity.

Since the discovery of the antitoxic property of the fluids of the body, it has been accepted that its manifestation was indispensable for the acquisition of immunity. It was thought that in order to get rid of pathogenic micro-organisms the animal had first to develop the means of neutralising their toxins. These substances once prevented from exerting their toxic action, the micro-organisms were left without their weapon of attack and found themselves reduced to the condition of simple saprophytes. It was accepted, therefore, that an effective antitoxic power was always to be found in the fluids of animals that had acquired immunity. Against this explanation, [304] however, are certain established facts. Chauveau[2] had observed that Algerian sheep, whose natural immunity was further strengthened by considerable doses of anthrax bacilli, exhibited a susceptibility to injections of anthrax blood quite as marked as that of normal sheep. The immunity against the virus, then, did not progress *pari passu* with that against the poison. Later, Charrin and

[1] *Compt. rend. Soc. de biol.*, Paris, 1899, p. 432.
[2] *Compt. rend. Acad. d. sc.*, Paris, 1880, t. xc, p 1526.

Gamaleia[1] furnished important data on this subject. They showed that animals vaccinated against the *Bacillus pyocyaneus* and the vibrios of Koch and Gamaleia were even more susceptible to intoxication by the soluble products of these micro-organisms than were normal animals which had acquired no immunity against the corresponding bacteria. Shortly afterwards this observation was confirmed by Selander[2], in his work on hog cholera, carried out under Roux's direction. Rabbits vaccinated against the cocco-bacillus of this disease resisted infection by the virus, but died as a result of the exhibition of the same doses of toxin that killed normal rabbits. I[3] was able not only to verify this, but to add to it the further fact that the blood serum of vaccinated rabbits, although markedly protective against infection, exercised not the slightest antitoxic action.

When, later, R. Pfeiffer set himself to study the immunity of animals against the cholera vibrio, he, along with his collaborators, was able to furnish numerous data confirming the hypothesis that animals thoroughly vaccinated against this vibrio had not thereby become more resistant to its toxin and that their anti-infective serum exhibited no antitoxic power. These results have been confirmed repeatedly and must be regarded as fully established.

Von Behring here recognised a general law which, with the aid of his collaborators, he attempted to develop. We owe to him the knowledge that the susceptibility, augmented as regards the toxins, of animals vaccinated against micro-organisms, might even serve in doubtful cases to reveal the presence of their bacterial poisons. Culture products when deprived of micro-organisms often set up no
[305] poisoning in normal animals susceptible to infection. From this fact it is generally concluded that the toxin is not present in the products in question. But animals of the same species when immunised against infection by the micro-organism, owing to their "hyper-susceptibility," react much more delicately and allow of the demonstration of the presence of bacterial poisons in fluids inactive for unvaccinated animals.

In collaboration with Kitashima[4], von Behring immunised guinea-pigs against the diphtheria bacillus, and demonstrated that two or three injections of diphtheria toxin were quite sufficient to render

[1] *Compt. rend. Soc. de biol.*, Paris, 1890, p. 294.
[2] *Ann. de l'Inst. Pasteur*, Paris, 1890, t. IV, p. 563.
[3] *Ann. de l'Inst. Pasteur*, Paris, 1892, t. VI, p. 295.
[4] *Berl. klin. Wchnschr.*, 1891, p. 157.

these animals refractory to infection by the diphtheria bacillus though they became more susceptible to intoxication. Von Behring considers that this augmentation of susceptibility to the diphtheria poison may be a means of rendering the local reaction of the living elements at the point of introduction of the bacilli more active.

In any case, it is beyond question that acquired immunity against microbial infection is quite independent of the resistance against the toxins of the corresponding micro-organism. An antitoxic manifestation of any kind, therefore, cannot be regarded as necessary for the development of immunity against the micro-organism.

Of all the humoral properties developed in acquired immunity against micro-organisms, the fixative power and the protective power are the most constant. It might naturally be suggested, as a result of this observation, that these two powers are indispensable for the manifestation of phagocytosis for the purpose of destroying and of ridding the animal of the pathogenic organisms. It is quite possible to understand how, under these conditions, the idea has been put forward that anti-infective acquired immunity is the result of two different factors: in the first place, a humoral property independent of the phagocytes and, in the second place, the phagocytes themselves. But the part played by these cells cannot be accepted as purely secondary—a view which has been advanced and defended again and again. This question is of such importance that it is reasonable to ask whence come the humoral properties, such as the fixative power and the protective power, factors of such far-reaching influence in anti-infective immunity?

Thanks to the work of several investigators this question may now [306] be answered. Pfeiffer and Marx[1] first supplied important information concerning the origin of the protective property. Into rabbits they made subcutaneous inoculations of cholera vibrios, killed by heat (70° C.), and then examined, most minutely, the protective power of the blood and of extracts from various organs. Examining, separately, the protective power of the serum and that of the layer of leucocytes deposited in tubes, Pfeiffer and Marx were unable to find any marked difference. Nor did they ever obtain any definite effect with leucocytes collected from pleuritic exudations. From these observations they concluded that the leucocytes of the blood could not be regarded as the source of the protective substance (or

[1] *Ztschr. f. Hyg.*, Leipzig, 1898, Bd. XXVII, S. 272.

"cholera antibody"). At a period when the serum as yet exhibited an insignificant protective power or none at all, the extract from the spleen often exerted an action of the most marked character. In an experiment in which the rabbit was killed 48 hours after the injection of the vibrios, 0·3 c.c. of the serum was incapable of preventing fatal infection of a guinea-pig, whereas 0·03 c.c. of an extract of the spleen exerted a marked protective effect. From this and similar experiments, Pfeiffer and Marx conclude that the spleen is the principal source of the protective substance. In order to verify this observation they injected killed cholera cultures into rabbits which had previously been deprived of their spleens, but the asplenic rabbits still produced the same amount of protective substance, and these two observers were led to conclude that the lymphatic glands and the bone-marrow might also serve as the sites of origin of this substance.

It is only during the first few days, however, that these organs exhibit a protective power greater than that of the blood. Three or four days after the injection of the vibrios the blood serum becomes richer in protective substance; the organs contain much less of it. This condition is maintained for some time, after which the blood in turn begins to get impoverished.

Pfeiffer and Marx put to themselves the question: Is the marked protective power of the spleen due to the production of preventive substance by this organ, or is it to be explained by an accumulation in the spleen of this substance manufactured elsewhere? With the
[307] object of obtaining an answer to this question they injected protective serum from other individuals into rabbits, when they found that the protective substance showed not the slightest tendency to accumulate in the spleen. These authors were compelled to conclude, therefore, that the spleen and other haematopoietic organs (lymphatic glands and bone-marrow) are the real seats of the production of the protective substance. We may add that these organs are also the phagocytic organs *par excellence*, that is to say, the centres which serve not only for the development of phagocytes but which contain a large number of the adult elements capable of exercising the phagocytic function.

Almost simultaneously with Pfeiffer and Marx, A. Wassermann[1], in collaboration with Takaki, undertook similar researches on the origin of the substance protective against the typhoid cocco-bacillus. The

[1] *Berl. klin. Wchnschr.*, 1898, S. 209.

outcome of this work was that "it was the bone-marrow, the spleen, and the lymphatic system, including the thymus gland, which exhibited immunising power against the bacillus of typhoid fever, whilst the other organs, the blood, brain, spinal cord, muscles, liver, kidney, etc., did not at this stage show any marked specific property."

As these observations on the production of protective substance in the phagocytic organs was one of essential importance in connection with the problem of acquired immunity, I asked M. Deutsch[1], working in my laboratory, to carry out a series of experiments on this subject. Using guinea-pigs, he injected into the peritoneal cavity cultures of the typhoid bacillus killed by heat (66° C.). A few days later the serum had become distinctly protective. At this stage, and even before the appearance of this property in the blood, Deutsch killed some of his animals and carefully measured the protective power of the extract of the various organs. He began by confirming the result obtained by Pfeiffer and Marx as to the non-production of the protective substance in the peritoneal exudation. Usually this fluid was insufficient to protect normal guinea-pigs against typhoid infection. In a few experiments only was the exudation found to be as protective as the blood serum ; in most of the others, the blood serum was much more active than the fluid of the exudation. The spleen was the organ which exhibited the greatest protective power, and [308] in nearly one half of the cases it was more active than was the blood. The bone-marrow sometimes gave analogous though much less marked results. The spleen consequently must be looked upon as the principal seat of the production of the protective substance.

Having confirmed this observation of Pfeiffer and Marx and of Wassermann and Takaki, Deutsch tried to obtain the protective property in guinea-pigs deprived of their spleens. The experiment was quite successful, and here again his result agreed with that obtained by Pfeiffer and Marx. Guinea-pigs from which the spleen had been removed developed the protective property just as well as did the control animals ; in the former the bone-marrow was found to be specially active.

When Deutsch, instead of removing the spleen from his guinea-pigs before the injection of the micro-organisms, did so some (3—5) days afterwards, there often occurred a marked diminution in the amount of the protective substance produced. We must conclude, therefore, that soon after inoculation there appears in the spleen

[1] *Ann. de l'Inst. Pasteur*, Paris, 1899, t. XIII, p. 689.

a phenomenon which is associated with the development of the protective power. The most simple explanation of these facts is that the micro-organisms injected into the peritoneal cavity and soon afterwards seized by the phagocytes (for the most part by the microphages), are carried to the phagocytic organs, particularly the spleen, lymphatic glands, and bone-marrow. In those animals whose spleens are left intact a large number of these microphages loaded with micro-organisms make their way into this organ, a fact confirmed by direct observation. When the spleen is removed the microphages must necessarily betake themselves to other phagocytic organs. As the micro-organisms undergo intracellular digestion in the phagocytes, it is very difficult, if not impossible, to follow them for any length of time after they have been ingested, but the analogy with the phenomena of the resorption of red blood corpuscles, described in Chapter IV, justifies us in concluding that in the case of micro-organisms matters go on in much the same way. These organisms, seized at the seat of inoculation by the phagocytes, are transported by these cells, in their peregrination through the organs, into the general circulation. The interpretation I have just given has been accepted by Deutsch.

This observer wished also to come to some conclusion as to the origin of the agglutinative property so well developed in the fluids of animals inoculated with the typhoid cocco-bacillus. He did not
[309] succeed in solving this question, but he was able to demonstrate the undoubted difference between this property and the protective power. The facts brought forward by Deutsch must, therefore, be ranged alongside the many others, reported on above, which demonstrate in the most conclusive fashion that these two powers of the body fluids are essentially distinct.

Such concordant results obtained by all investigators who have studied the origin of the protective power warrant the conclusion that it is the elements of the phagocytic organs, that is to say, the phagocytes themselves, which produce the protective substance. But it will be asked : Can we therefore accept the fixative substance or fixative as being derived from the same source? When the experiments I have just summarised were carried out the fixatives were not as yet sufficiently known and were confounded with the protective substances. Nevertheless, there can be no doubt as to what the answer to the question just put must be. In the account of the experiments of Pfeiffer and Marx we find very precise state-

ments as to the granular transformation of the vibrios. Thus, they observed on several occasions that an extract of the spleen set up this transformation in a particularly distinct and rapid fashion at a period when the blood and serum, used in a much stronger dose, were incapable of producing the same effect. Now, as Pfeiffer's phenomenon is a visible manifestation of the action of the specific fixative, it cannot be doubted that the spleen is really the principal seat of development of the fixative substance before it makes its appearance in the blood.

Before concluding this chapter we must review very briefly the principal phenomena associated with acquired immunity against micro-organisms. The extracellular destruction of these parasites takes place in the living animal under special conditions only, when the phagocytes suffer a temporary injury (phagolysis) and allow their microcytases to escape. These latter by no means represent attributes of the body fluids, as is even yet maintained by some writers. These soluble ferments are connected with the phagocytes and represent the ferments of intracellular digestion. The cytases undergo no modification during the process of immunisation and correspond to those which act in natural immunity.

The agglutinative substance often present in the normal fluids of the body becomes much more developed in those of immunised animals. It is truly humoral, as it circulates in the plasmas and passes into the fluid exudations and transudations. But the part played by [310] it in immunity is very restricted.

The protective and fixative properties, most often closely connected with each other, are very markedly developed in an animal enjoying acquired immunity. They may act upon the micro-organisms which become permeated by the fixative substance, or upon the infected animal by stimulating its defensive reaction, but they are incapable of affecting the vitality or virulence of the micro-organism. The two properties (protective and fixative) reside in the fluids of the body, but they are functions of the cell products. The elements of the phagocytic organs (spleen, bone-marrow, lymphatic glands), or phagocytes, produce the specific protective and fixative substances which pass thence into the plasmas.

The phagocytic reaction is very general in acquired immunity. The phagocytes which have a very imperfect antimicrobial function or none at all, become, as the result of vaccination, much more active. They exhibit a very marked positive chemiotaxis and

acquire the faculty of digesting micro-organisms in a greatly intensified degree. It is with the increase of this digestive power that we have connected the over-production by the phagocytes of the fixative and protective substances which are excreted in large quantities by these cells and pass into the fluids of the animal. As these substances are phagocytic products it may be readily conceived that in certain examples of acquired immunity the animal overcomes the micro-organisms without the protective substances being found in the fluids. It is sufficient that it is in the possession of the phagocytes, which may retain it within themselves and not throw it off into the circulation.

From this account it will be seen that the phenomena, in acquired immunity against micro-organisms, are merely a more or less stereotyped copy of those that are presented in the animal after the resorption of cells. There, also, we have intracellular digestion with over-production of specific fixatives, part of which are excreted and thus pass into the plasmas. In the resorption of cells there is also a double action of cytases and fixatives; but in this case the macrocytases intervene, whilst in the resorption of micro-organisms this function is performed by the microcytases. The fixatives in the two cases are very different from the point of view of their action, for
[311] they are specific; but the cells which act in their production belong, in both cases (resorption of animal cells and of micro-organisms), to the category of phagocytes.

It is often maintained that the theory I have just summarised is fundamentally opposed to the theory of side-chains or receptors formulated by Ehrlich[1]. This view I cannot accept. Applied to acquired immunity against micro-organisms this theory may be summed up as follows. The micro-organisms, when inoculated in a non-lethal but immunising dose, combine with certain cells of the animal. The receptors of the micro-organisms find corresponding receptors in these cells, but, when once combined, the receptors of the cells become incapable of fulfilling their normal nutritive function. The cells, thus deprived of their receptors, reproduce such an enormous quantity of them that a portion is excreted into the surrounding medium and passes into the plasmas. These receptors, originating from cells, but which have become constituent parts of the body fluids, are nothing but the fixatives or intermediary bodies,

[1] Ehrlich, Lazarus u. Pinkus, "Leukaemie, etc." in Nothnagel's "Specielle Pathologie u. Therapie," Wien, 1901, Bd. VIII, I Theil, III Heft, Schlussbetrachtungen, S. 163.

or the amboceptors of Ehrlich. On a fresh arrival of the same micro-organisms, they meet with, in the fluid of the exudations, numerous amboceptors which combine with the corresponding receptors of the micro-organisms, without, however, destroying them or interfering with their vitality. As these amboceptors possess still a second affinity, that for the molecules of the cytases, or the "complements" of Ehrlich, the micro-organisms can be placed in contact with these soluble ferments. Without the intervention of the fixatives, the combination of the body of micro-organisms with the cytase can never take place, because the receptors of the micro-organisms are not adapted to those of the cytases. When the molecules of these ferments are found in the plasmas in a free state, they can be attacked by the corresponding group of the amboceptors.

Let us compare the theory we have just sketched with that described further back. The micro-organisms, inoculated with a non-lethal but immunising dose, are, as we have seen, ingested by the phagocytes and afterwards digested within them. This intracellular digestion is followed by the over-production of the specific fixative, of which a part is excreted and passes into the plasmas. These are the results of the well-established experimental data [312] described in this chapter. Ehrlich's theory is in no way in opposition to this; it simply attempts to penetrate more deeply into the mechanism of the phenomena observed as taking place between the micro-organism and the cell. The act which we simply term intracellular digestion is divided by Ehrlich into its constituent parts. According to him there is a combination of the fixative, on the one hand, with the molecule of the micro-organism, on the other, with that of the soluble ferment or cytase. According to Ehrlich it is the amboceptors of the cells which become detached in order to furnish the fixative that circulates in the plasmas. For us there is simply an over-production of one of the two ferments of intracellular digestion, without defining more exactly what constituent part of this ferment passes into the circulation. The two theories may supplement each other but are in no way contradictory in principle. There is only a single important point wherein they do not accord. Ehrlich thinks that the cytases are always free in the body fluids and that the cells, in order to exert a digestive action on the micro-organisms, must previously seize their molecules by means of one of the groups of their amboceptors. We, on the contrary, have developed the idea that the

cytases are only free in the animal during phagolysis and that under normal conditions the cytases remain closely bound up with the phagocytes. This statement is based upon a large number of well-established experimental facts and must therefore be accepted as proved. It does not, however, affect any fundamental principle of Ehrlich's theory. On the other hand the bases of Ehrlich's theory affect none of the main features of the theory I have developed. The doctrine which regards acquired immunity as a particular case of resorption may be reconciled with the conception of amboceptors. But it accords equally well with Bordet's conception, according to which the fixatives act not as intermediary substances between the micro-organism and the cytase, but as substances which sensitise the micro-organisms for the penetration of the digestive ferment. This delicate question has not yet been definitely settled, but Bordet's experiments described in Chapter IV are greatly in favour of this view.

Neisser and Wechsberg[1] have tried to obtain some idea of the
[313] manner in which the fixatives act on the micro-organisms and have recorded a series of very interesting facts. They have shown that these substances only bring about the destruction of bacteria when they are in certain relations with the cytase. Mixtures of fixatives and cytases in which the former are found in excess not only do not kill the micro-organisms but even allow them to develop abundantly. To attain this result Neisser and Wechsberg mixed constant quantities of bacteria and normal serum containing cytase with variable quantities of the serum of immunised animals heated to 56° C. As we know, this specific serum, as the result of being thus heated, is deprived of its cytases, but may be readily made active again by the addition of normal, unheated serum. This paradoxical fact, demonstrated by Neisser and Wechsberg can, in their opinion, be explained only by Ehrlich's theory of amboceptors. When these bodies with double affinities are found in too large quantity as regards the cytase, it may happen that one part only of those which combine with the receptors of the micro-organisms succeed in linking to themselves the molecules of the active ferment. The amboceptor being by itself incapable of destroying the micro-organism, can be injurious to it only on condition that it brings cytase. Consequently as the amount of this cytase is too small for the much larger number of amboceptors we can readily conceive that the micro-organisms may profit

[1] *München. med. Wchnschr.*, 1901, p. 697.

thereby and remain alive. This interpretation is certainly very ingenious, but nothing proves that it corresponds with the real state of things. Neisser and Wechsberg have themselves observed that the serum of the normal goat can also prevent the bactericidal action of the cytase. In this case, however, they suggest the intervention of an anticytase of this normal serum. The same explanation might perhaps serve also to explain the preventive action of the serum of immunised animals. We know that anticytases are found frequently enough in the various serums and that they undergo great variations, according to the conditions present in the animals furnishing the blood.

In any case, it is evident that the theory of receptors must in no way be regarded as the antithesis of the theory of phagocytosis. This latter quite retains its right to affirm that, in acquired immunity against micro-organisms, phagocytes play the most general and important part. They hold back the cytases which are capable of ridding the animal of micro-organisms from destroying them. It is further these same cells that produce and excrete the [314] fixative and protective substances. The free fixatives may attack the micro-organisms in the body fluids but they are incapable of depriving them of life or even of virulence. The cytases, after escaping from the phagocytes, may certainly, in collaboration with the fixatives, destroy a certain number of the micro-organisms, but only in special cases met with, no doubt, but only rarely, under natural conditions. On the other hand, the phagocytes in the animal which enjoys acquired immunity constantly fulfil the function of seizing the micro-organisms and of submitting them in their interior to the combined action of fixatives and cytases.

Acquired immunity, like natural immunity against micro-organisms, presents merely special phases of intracellular digestion.

CHAPTER X

[315] RAPID AND TEMPORARY IMMUNITY AGAINST MICRO-ORGANISMS, CONFERRED BY SPECIFIC AND NORMAL SERUMS, OR BY OTHER SUBSTANCES, OR BY MICRO-ORGANISMS OTHER THAN THOSE AGAINST WHICH IT IS DESIRED TO PROTECT AN ANIMAL

Immunity conferred by specific serums.—Analogy of the mechanism of this immunity with that observed in immunity obtained with pathogenic micro-organisms and their products.—Part played by phagocytosis in the immunity conferred by specific serums.—Influence of opium on the course of immunisation by these serums.—Stimulant action of specific serums.—Protective and stimulant action of normal serums.—Influence of fluids, other than serums: broth, urine, physiological saline solution, etc.

Antagonism between anthrax and certain bacteria.

We have seen how important in the study of acquired immunity against micro-organisms is the demonstration of the protective power of the body fluids. Without being absolutely general, this power is, nevertheless, widely diffused and is found even in certain examples of acquired immunity against micro-organisms belonging to the animal kingdom. Up to the present I have refrained from doing more than point out the presence, in the fluids of the immunised animal, of this protective property and have studied it exclusively in relation to the animal that produces it. We must now pass to the question: How do the protective substances act in the animal which receives them ready formed? This immunity, which has received from Ehrlich the name of *passive immunity* against micro-organisms, must now be examined.

The study we now propose to enter upon is rendered much easier from our study of the data acquired on the phenomena exhibited in the animal vaccinated with micro-organisms or their products, data already given in the preceding chapter. There is, indeed, a very striking analogy between the two kinds of immunity, and though we draw a sharp line of distinction between them, this is due to the fact

that the immunity conferred by micro-organisms or their products requires some time for its development and endures for a long period, [316] whilst the immunity due to the introduction of specific serums into an animal is set up immediately, but endures for a very short time only.

The diseases of the Invertebrata being seldom due to the micro-organisms that produce infections in the higher animals, the question as to whether the Invertebrata can be immunised by means of protective serums has not yet been studied. Still, we already have certain ideas on the protection of lower vertebrates by specific serums. Gheorghiewsky[1], in my laboratory, carried out some experiments on this point. He found that the serum of mammals (guinea-pig, goat) immunised against the *Bacillus pyocyaneus*, was under certain conditions capable of protecting the green frog against a dose of this organism that was always fatal to the control animals. When injected along with the *Bacillus pyocyaneus*, the serum did not prevent a fatal infection ; sometimes this infection developed even more rapidly than in the control frogs. It was only when the protective injection was made 24 or, better still, 48 hours before the inoculation of the bacilli, that the protective action became evident. The serum used in these experiments was not bactericidal for the *Bacillus pyocyaneus* which grew most luxuriantly ; but it agglutinated a large proportion of the bacilli. Gheorghiewsky pointed out, however, that frogs injected with cultures agglutinated by the goat's serum died just as readily as did the control animals. As the phagocytic reaction was invariably very active in those frogs which resisted the virus, after the injection of protective serum, it is very probable that this fluid exercises a stimulant influence on the phagocytes.

This idea of stimulation by anti-infective serums in cases of temporary immunity conferred by these fluids, has already been set forth in my researches on the immunity of rabbits against the Gentilly cocco-bacillus, induced by the serum of vaccinated rabbits. This view, however, has not been favourably received, especially in view of the discovery of the phenomenon of the transformation of cholera vibrios into granules. Pfeiffer himself noted that this transformation took place not only in the peritoneal cavity of vaccinated guinea-pigs but also in the peritoneal cavity of normal guinea-pigs, into which he [317] had injected small quantities of specific serum. As this latter fluid, in Pfeiffer's hands, was incapable of transforming the vibrios into granules *in vitro*, he concluded that the cellular elements of the

[1] *Ann. de l'Inst. Pasteur*, Paris, 1899, t. XIII, p. 315.

normal animal were endowed with the power of modifying the inactive substance of the specific serum into bactericidal substance. According to this conception the immunity conferred by this serum was not entirely passive since, in order to prepare the substance which transforms and kills the vibrios, the co-operation of the living cells was necessary.

My demonstration of the possibility of obtaining Pfeiffer's phenomenon *in vitro* at once turned the balance in favour of the theory that the immunity induced by the specific serum is due to a direct humoral action upon the micro-organism. Under these conditions such immunity could only be interpreted as being purely passive. This view seemed to be finally established by Bordet's discovery that a specific serum, inactive by itself, became capable of producing Pfeiffer's phenomenon, as soon as a small quantity of normal, non-specific serum was added to it. Bordet[1] thus sums up his theory of the immunity conferred by specific serums: "Passive immunity is due, in part at least, to a chemical action exerted on the vibrios by two pre-formed substances, the one present in the animal before any injection is made, the other found in the serum that is injected; this phenomenon is purely chemical in the sense that it can go on without the aid of a vital reaction, of any new cell secretion: indeed it is found to take place in fluids from which the cells have been entirely removed" (p. 217). These demonstrations led up to the belief that the organism of the animal remained absolutely passive when it was subjected to the action of protective or anti-infective serums, and that the case of the cholera vibrio represented a kind of schema, which was applicable to the whole of the group of phenomena met with in passive immunity.

As in the study of the immunity obtained as the result of vaccinations with micro-organisms or their products, so in "passive immunity" there was seen only the direct chemical action of two substances on the micro-organism, and efforts were made to extend this demonstration to a series of infective diseases.

[318] Pfeiffer and Kolle[2] having observed that the blood serum of persons convalescent from typhoid fever, as well as that of animals vaccinated with the typhoid bacillus, exhibited a great protective power for the guinea-pig, wished to get some idea of the mechanism of this immunity. They found that in the peritoneal cavity of guinea-

[1] *Ann. de l'Inst. Pasteur*, Paris, 1896, t. x, p. 193.
[2] *Ztschr. f. Hyg.*, Leipzig, 1896, Bd. xxi, S. 203.

pigs, inoculated with the typhoid cocco-bacillus and simultaneously subjected to the action of protective serums, the micro-organisms lose their mobility almost immediately. A little later, they exhibit a degeneration of form, become less refractile and disintegrate. After the injection of large doses of specific serum the bacilli, much as in the case of the cholera vibrio, become transformed into granules. "But," say these authors, "this last mode of destruction, that is to say the formation of granules at the expense of the injected bacteria, does not occur with such remarkable regularity as it does in Pfeiffer's phenomenon in the cholera vibrio" (p. 219). Whilst these changes are going on in the peritoneal fluid, the leucocytes begin to come up and to ingest the bacilli and their *débris*. "Phagocytosis, therefore, undoubtedly plays a part in the destruction of the bacteria. Nevertheless, as most of the injected bacteria die in the fluid of the exudation, phagocytosis can not be regarded as the cause of the protective action of the serum" (p. 220). We see from this description that even in the case of the typhoid cocco-bacillus the direct action of the fluids of the body is perceptibly less marked than in the case of the cholera vibrio. Even in the latter, however, it is necessary to make many reservations. The same laws apply to the immunity against this micro-organism, conferred by the serum of immunised animals, as to the immunity due to vaccinations by the vibrios or their products. To treat this subject fully one would have to repeat almost textually the two preceding chapters, but I will simply recall the fact that this transformation, almost general and very rapid, as we observed *in vitro* in vibrios placed in contact with fresh specific serum or with the mixture of this serum, heated to 55°—56° C., and normal unheated serum, is only met with in the animal body where phagolysis appears. Pfeiffer first observed the phenomenon which bears his name in the peritoneal cavity, and it is best seen in that situation during the period of the phagolysis of the white corpuscles. Vibrios, mixed with small doses [319]
of specific serum which by itself is able to render them motionless and agglutinate them, but which is absolutely unable to transform them into granules, present this transformation immediately they are introduced into the peritoneal cavity of normal guinea-pigs. In this case the vibrios, permeated by the fixative of the specific serum, are affected by the microcytase which has escaped from the injured phagocytes and is found in the peritoneal fluid. The preparation of the peritoneal cavity of normal guinea-pigs by means of an injection

of broth or physiological saline solution the day before, prevents the production of Pfeiffer's phenomenon, in spite of the protective serum, just as in vaccinated guinea-pigs. In both cases the vibrios, without being transformed into granules by the fluid part of the peritoneal exudation, are ingested by the phagocytes *en masse* and with extraordinary rapidity. This experiment was repeated by Garnier[1] with the typhoid cocco-bacillus. He first injected into the peritoneal cavity of young guinea-pigs several c.c. of physiological salt solution, of fresh broth or of some other fluid. The next day he introduced into the same situation typhoid cocco-bacilli mixed with blood serum from a donkey that had been for a long time immunised against this organism. A few minutes (2—4) after this latter injection the leucocytes, whose phagolysis had been prevented by the previous day's preparation, were found crammed with cocco-bacilli. Some of these bacilli, like those still free in the peritoneal fluid, retained their normal form, but a very large number of those ingested by the microphages were already transformed into granules. This experiment affords fresh confirmation of the hypothesis that the substance which transforms the cocco-bacilli or the vibrios into granules is the microcytase. In the phagocytes in their normal condition this microcytase is found in the microphages, but during phagolysis a portion of it escapes into the surrounding fluid. In the control experiments made by Garnier with young normal guinea-pigs not prepared by preliminary injection, the simultaneous injection of typhoid cocco-bacilli and specific donkey's serum set up this attenuated and not very typical Pfeiffer's phenomenon described in Pfeiffer and Kolle's memoir.

Soon after the discovery of Pfeiffer's phenomenon I[2] was able to bring forward a proof that it was produced neither in the sub-
[320] cutaneous tissue, in the oedemas set up by the arrest of the circulation, nor in the anterior chamber of the eye of animals when cholera vibrios mixed with anti-infective specific serum were injected into these situations. Here the micro-organisms retain their normal form, remain quite alive and in this condition are ingested by the leucocytes which are brought up to the points invaded. These cells, attracted by the vibrionic products, do not undergo any phagolysis and, untrammelled, fulfil their phagocytic function. Inside them are found vibrios which have kept their elongated form and others which have become transformed into granules. The exudations containing

[1] *Ann. de l'Inst. Pasteur*, Paris, 1897, t. XI, p. 773.
[2] *Ann. de l'Inst. Pasteur*, Paris, 1895, t. IX, p. 453.

these elements still give cholera cultures on nutrient media, a proof that some at least of the intracellular vibrios are alive. Here we have no destruction of the micro-organisms in the fluids of the body, consequently no direct action of the bactericidal substance. This substance, enclosed in the phagocytes, can only act through the intervention of these elements.

Mesnil[1] made analogous experiments with the Massowah vibrio, which, unlike the cholera vibrio, is peculiarly virulent when injected subcutaneously into guinea-pigs. In spite of this difference, this micro-organism, when injected along with protective serum into normal animals, behaves much as does the cholera vibrio proper. Mesnil injected the Massowah vibrios at the same time as the anti-infective specific serum, into the subcutaneous tissue of young and adult guinea-pigs and of young rabbits. In every case he observed the same reaction phenomena in the animal organism. The vibrios caused the formation of oedema at the point of inoculation and remained isolated in the fluid. The majority of these micro-organisms became motionless, but a few remained motile. Pfeiffer's phenomenon was never observed. There was sometimes an aggregation of the vibrios, but this was not comparable with the marked agglutination brought about by the specific serum *in vitro*. The vibrios retained their power of reproduction, and Mesnil was able to observe them in all phases of division. Some hours (6—8) after inoculation the leucocytes began to come up to the seat of injection and set to work at once to ingest the vibrios. This phagocytosis became more and more marked, and finally there was ingestion of the whole of the micro-organisms. Drops of the exudation containing only intraphagocytic vibrios, when placed [321] in the incubator, gave abundant cultures. The leucocytes died outside the animal body, whilst the vibrios continued to live and grow well under the new conditions. Certain leucocytes became three times their original size, and their contents were seen to be made up of vibrios closely packed together. The subcutaneous exudation, when withdrawn even eight days after the injection of the micro-organisms and sown on nutrient media, still gave colonies of vibrios.

It is evident, therefore, that the direct action of the protective serum on the vibrios was reduced to a mere trifle. It rendered them motionless and brought about a very slight clumping, but it was incapable of transforming the vibrios into granules or of destroying them. We see, then, that even in the case of the vibrios, the part

[1] *Ann. de l'Inst. Pasteur*, Paris, 1896, t. x, p. 371.

played by Pfeiffer's phenomenon is very limited. The destruction of the vibrios is effected with certainty, and completely, under the influence of the specific serums, not by a direct action of the two anti-bacterial substances but through the mediation of the phagocytes. Before the fixative, introduced with the protective serum, can bring about this result, the leucocytes, impressed with a special sensitiveness, must come up to the seat of inoculation, seize the micro-organisms and secrete around them their cytase. It is only as a result of these actions, purely vital, that the chemical or physico-chemical reaction of the substances which intervene in the destruction of the vibrios is brought about.

Under these conditions it can easily be understood that if the vital action of the phagocytes is retarded or depressed the injection of protective serum cannot preserve the life of the animal. Cantacuzène[1], who had already made a similar demonstration on guinea-pigs vaccinated against the cholera vibrio by these organisms or by their products, carried out numerous experiments on the action of opium on normal guinea-pigs simultaneously inoculated with vibrios and specific serum. Before injecting this mixture Cantacuzène narcotised his animals by means of tincture of opium. The great majority ($\frac{4}{5}$) of the guinea-pigs so treated died at the end of one or several days. The transformation of the vibrios into granules, under the influence of the serum, took place in the peritoneal cavity, but the leucocytes, on account of the narcotic action of the opium, were tardy in coming up. On their arrival in the peritoneal cavity they were capable of
[322] ingesting the granules, but absolutely refused to seize entire vibrios, always fairly numerous in the exudations. In spite of the appearance of a large number of leucocytes, these cells were still too weak to offer any adequate opposition to the vibrios, which increased in number and continued to multiply up to the death of the animal, when the exudation simply swarmed with very motile vibrios. Sometimes the struggle was prolonged. The weakened leucocytes allow the vibrios to develop, but, after a greater or less length of time, they regain their strength and begin to ingest the micro-organisms vigorously. Complete phagolysis follows, but the guinea-pig, attacked by the toxic products of the vibrio, finally succumbs in spite of the absence of free vibrios from its body.

An analysis of the phenomena observed in the body of an animal treated with antivibrionic serum, demonstrates that, in spite of a

[1] *Ann. de l'Inst. Pasteur*, Paris, 1898, t. XII, p. 290.

certain direct action of the substances contained in this fluid, there still remain a whole series of processes, amongst which the carriers of the cytases, that is to say the phagocytes, fill the most important *rôle*. Nevertheless, the cholera vibrio with its allied forms is still the most sensitive of all the micro-organisms to the bactericidal action of the fluids of the body. It may, therefore, readily be conceived that the more resistant micro-organisms are even less subject to the direct influence of the specific serums. Thus we have seen that the cocco-bacillus of typhoid fever presents, in the phagolysed peritoneal fluid, merely an attenuated form of Pfeiffer's phenomenon. The other representatives of the group of bacilli are still less subject to the direct action of the serums, and Gheorghiewsky[1], in his studies on the *Bacillus pyocyaneus*, found that normal guinea-pigs, injected subcutaneously with anti-infective specific serum, and inoculated into the peritoneal cavity with this organism, present the same phenomena as those described in Chapter VIII. He never noticed either lysis of the bacteria in the fluids of the animal or their total transformation into agglutinated masses outside the phagocytes. The resistance offered by the animal was always in direct relation to the rapidity of the appearance and extent of the phagocytic reaction.

In order to determine the relative importance of each of the factors which act in the preservation of animals subjected to the influence of the specific serum, Gheorghiewsky repeated Cantacuzène's experi- [323]
ments on the effect of narcotisation by tincture of opium. This alkaloid retards diapedesis, but does not affect the tactile sensibility or the motility of the leucocytes. The humoral properties, on the other hand, are not in the least affected by the narcosis. In spite of the fact that in guinea-pigs, narcotised and treated with anti-infective serum, the direct action was not interfered with, the animals always died because the retarded and incomplete phagocytic reaction was insufficient to destroy the bacilli rapidly enough.

Mesnil[2] studied the action of the specific serum against swine erysipelas on normal animals into which he had injected it some time before inoculation of the corresponding bacillus into the peritoneal cavity. This serum exercises a most marked protective action on the mouse, an animal very susceptible to the pathogenic action of this micro-organism. In mice so prepared complete and rapid phagocytosis takes place. These micro-organisms before being ingested

[1] *Ann. de l'Inst. Pasteur*, Paris, 1899, t. XIII, p. 312.
[2] *Ann. de l'Inst. Pasteur*, Paris, 1898, t. XII, p. 492.

by the phagocytes show no appreciable change; they are always stained very uniformly and intensely by Gram's method, and they never swell up. The bacilli undergo no agglutination in the body of the mouse, a fact of which we can convince ourselves by examining hanging drops of the exudation. The phenomenon which strikes the observer most is the very pronounced phagocytosis, due principally to the activity of the microphages. Some hours after inoculation these cells are found to be crammed with bacilli, a large number of which no longer stain in the normal fashion. Without being transformed into granules, these micro-organisms undergo intracellular digestion which at the end of a few days is complete. This destruction is more rapid and complete in the microphages, slower in the macrophages. Drops of exudation collected from these mice, at a stage when the ingestion is completed, produce fatal septicaemia in untreated mice. This is proof that at the moment when they were seized by the phagocytes the bacilli still retained their virulence. Mesnil, as the result of his experiments, concludes that "the effect of the serum is to stimulate the phagocytes and especially the polynuclear forms; they ingest more quickly, they digest more quickly. The serum is, therefore, a stimulant of the cells charged with the defence of the animal" (p. 496).

[324] We need not describe the phenomena produced in mice inoculated subcutaneously and treated with protective serum, for even in the peritoneal cavity neither Pfeiffer's phenomenon nor any extracellular destruction of the bacilli can be observed. The micro-organisms, when subjected to the influence of the specific serum, readily absorb the fixative, as demonstrated by Bordet and Gengou[1]. This absorption must certainly favour the action of the intraphagocytic cytases. It is not, however, sufficient to explain the protective, anti-infective action of the serum. Such explanation was given by the experiments which Gengou, at my request, was good enough to make. He inoculated mice with the bacilli of swine erysipelas, mixed with specific serum heated to 55° C., to which was added some normal guinea-pig's serum. The mice so treated resisted the infection but controls died in a few days. Being thus assured of the protective action of the serum, Gengou prepared the same mixtures of swine erysipelas bacilli and of the two serums; but, instead of injecting the whole of the mixture, he removed the bacilli from the serums, after a prolonged contact, and injected the bacilli alone into the mice. The

[1] *Ann. de l'Inst. Pasteur*, Paris, 1901, t. xv, p. 289.

bacilli had become permeated with fixatives, but, in spite of this, they killed the mice almost as quickly as the controls. Consequently, it is not the fixative adherent to the micro-organisms which determines the protective action of the specific serum. This fluid must contain another substance, one that will stimulate the phagocytes.

The analysis of the mechanism of the immunity termed passive, that is to say, communicated to normal animals by the introduction of an anti-infective specific serum, teaches us that, even when the direct action of the humoral substances is very limited, the protective effect, thanks to the stimulant action which brings about the destruction of the micro-organisms through the mediation of the phagocytic reaction, is still produced. The result at which we have thus arrived is confirmed by the examination of the phenomena observed in animals subjected to the action of anti-anthrax serum. Marchoux[1] first supplied us with precise details as to the mode of action on the rabbit of the serum of animals treated with anthrax bacilli. He found that, in the peritoneal cavity of rabbits injected the day before with anti-anthrax serum, the inoculated anthrax bacilli almost immediately become the prey of [325] phagocytes. Within a couple of minutes after the introduction of bacilli into the peritoneal cavity, the great majority of them are ingested by the leucocytes; ten minutes later, there are no free bacilli. Not only the ingestion but also the destruction of these micro-organisms takes place with great rapidity, and even a few hours after the injection, the peritoneal exudation, when sown on nutrient media, remains sterile. In the subcutaneous tissue the phagocytic reaction requires a longer time than in the peritoneal cavity, nevertheless, it goes on very rapidly. Thus, when inoculated into the subcutaneous tissue of the ear of rabbits treated with specific serum, the bacilli are in great part ingested at the end of half-an-hour. At the end of an hour phagocytosis is usually complete.

In Marchoux's experiments the importance of the part played by the phagocytes becomes still more prominent when it impedes their function in any way. Rabbits injected with anti-anthrax blood and 24 hours later inoculated below the skin of the ear with anthrax bacilli always resist infection, exhibiting the well-marked phagocytosis just mentioned. In other rabbits, however, prepared in the same way with the serum, but inoculated the following day into an ecchymosis excited by tapping the ear lightly, a certain number of the bacilli escape the phagocytes and succeed in setting up an

[1] *Ann. de l'Inst. Pasteur*, Paris, 1895, t. IX, p. 800.

abundant oedema followed by a fatal anthrax at the end of a few days. On making a post-mortem examination of these animals the bacilli were not numerous, but they were found in all the organs. The same result was obtained in another experiment in which Marchoux inoculated subcutaneously with anthrax blood which coagulated *in situ* rabbits prepared with specific serum. The blood clot attracted only the macrophages, as pointed out in Chapter IV. The microphages did not come up until late and then in small numbers. Now, as these are the phagocytes that are chiefly instrumental in destroying the anthrax bacillus, their absence allowed the bacilli to multiply and to set up a fatal anthrax. The rabbits prepared with the same serum but injected with anthrax blood diluted with broth (which prevents the formation of clot) completely resisted infection, thanks to the phagocytic reaction which went on without hindrance.

Sclavo[1] also, who made numerous investigations on the action
[326] of the anti-anthrax serum, is of opinion that this action is not a direct one upon the bacillus but is produced indirectly through the action of the animal organism. He maintains that the serum stimulates the function of the phagocytes and augments the bactericidal action of the body fluids. But since this bactericidal power enters the cytase as a substance destroying the micro-organisms, and this cytase is contained in the phagocytes, we can readily understand what a dominant part in the process these elements play.

Sobernheim[2], also, has paid much attention to the question now under discussion. As the result of his researches he comes to the conclusion that the anti-anthrax serum "cannot exert any effect on the virus by a direct action of the protective specific substances." In order that the serum may be effective, the active intervention of the organism of the animal is necessary, otherwise, it is impossible to explain why the serum, used in the same proportion against the same quantity of anthrax bacilli, should protect one species of animals (the rabbit) and allow another (guinea-pig, mouse) to succumb. When Sobernheim tried to apply to anthrax the discovery of the transformation of cholera vibrios into granules, he got only negative results. There was nothing produced comparable to Pfeiffer's phenomenon and the anthrax bacilli usually underwent no apparent modification. Sobernheim affirms also that the rapid phagocytosis under the influence of the serum, described by Marchoux, "does not

[1] *Centralbl. f. Bakteriol. u. Parasitenk.*, Jena, 1899, I Abt., Bd. XXVI, S. 428.
[2] *Ztschr. f. Hyg.*, Leipzig, 1899, Bd. XXXI, S. 110.

appear to be produced under all circumstances" (p. 117). As, however, his researches on this subject were made on guinea-pigs which, in spite of the treatment with specific serum, always ended by succumbing to anthrax, we readily understand that his results cannot be compared with those obtained by Marchoux. I was present at the experiments of this observer and convinced myself of the accuracy of the facts recorded in his memoir.

Most of the examples here studied justify fully the hypothesis of the stimulant action of protective serums, a view that I formulated as the result of my researches on the immunity of rabbits against the Gentilly cocco-bacillus[1]. In this the first case of anti-infective immunity, due to the serum elaborated by an immunised animal, I could not find either a bactericidal action, however slight, or any agglutinative or attenuating property of the fluids of the body. As, on the other hand, this serum had no antitoxic power, [327] everything indicated that we must look for its action, which was *nil* or very slight on the micro-organism, as being exerted on the organism of the animal into which it was injected for protective purposes. A comparative examination of the course of the phenomena in the subcutaneous tissue of the ear in rabbits, some of which received an injection of the specific serum into the veins whilst others were kept as controls, at once showed how widely different were the two cases. In the control animals, the cocco-bacilli immediately began to multiply without meeting with any opposition on the part of the organism of the animal; on the other hand, in the rabbits treated with serum, the serum became rich in leucocytes which at once set to work to ingest the micro-organisms. In course of time the latter gradually diminished in numbers, whilst the leucocytes went on increasing. The phagocytosis, also, became more and more marked. This struggle was continued for more than 24 hours, after which the purulent exudation, containing masses of leucocytes, no longer included any cocco-bacilli visible under the microscope either outside or inside cells. Nevertheless, this pus was still capable of producing a fatal septicaemia in untreated rabbits, clearly proving that it still contained some living and virulent micro-organisms. These cocco-bacilli persisted for a long time inside the phagocytes; their presence being demonstrated by injecting the exudation into unprotected rabbits and thus setting up a fatal infection. Finally, however, they disappear completely. On consideration of such facts

[1] *Ann. de l'Inst. Pasteur*, Paris, 1892, t. VI, p. 308.

as these I considered that I was justified in formulating the following conclusion at the end of my memoir: "From the facts I have described, taken collectively, we may draw the conclusion that the preservation of unvaccinated rabbits treated with serum is due to a superactivity of the phagocytic defence; and it is allowable to express the opinion that the protective serum of hog cholera acts in rabbits by stimulating the phagocytes, rendering them less sensitive to the toxins, and by stimulating them in their struggle against the bacteria" (p. 310). The facts since collected by various observers fully justify this hypothesis. Amongst the other micro-organisms against which a rapid immunisation has been obtained by means of serum, we must cite the cocco-bacillus of bubonic plague. Numerous experiments, carried out on several species of animals, have shown that antiplague serum markedly augments the phagocytic reaction.

[328] In the group of the cocci, the streptococci have been specially fully studied from the point of view now under discussion. As already stated in another chapter, success has been attained not only in thoroughly immunising several species of animals against this dreaded micro-organism but active serums have been obtained capable of conferring distinct and certain immunity. The protective action of Marmorek's serum, prepared at the Pasteur Institute, has been specially carefully studied. This serum is obtained from horses that have received numerous injections of various races of streptococci pathogenic for animals and for man[1]. At Louvain, Denys and his pupils prepared several other antistreptococcic serums and studied their protective effect on laboratory animals.

In collaboration with Leclef, Denys[2] began by vaccinating rabbits against streptococci and studied the mechanism of the immunity obtained in these animals. A summary of their researches will be found in the eighth chapter. Denys and Leclef considered that the serum of vaccinated rabbits intervenes in two ways, first by directly hindering the multiplication of the streptococcus and then by exalting the activity of the leucocytes. They applied these results to the case in which immunity is conferred upon normal rabbits by the intervention of the serum of the vaccinated rabbit, but they were unable to furnish any data bearing directly on this immunity. Somewhat

[1] Marmorek, *Ann. de l'Inst. Pasteur*, Paris, 1895, t. IX, p. 593.

[2] *La Cellule*, Lierre et Louvain, 1895, t. XI, p. 175, and *Bull. Acad. roy. de méd. de Belg.*, Bruxelles, 1895.

later, Denys[1], in collaboration with Marchand, published another memoir in which he describes experiments on the mechanism of the immunity conferred on rabbits by injections of the blood-serum of vaccinated horses. From these experiments they draw the conclusion that "the serum of the horse immunised against the streptococcus possesses no bactericidal properties, properly so called, against this micro-organism; it does not affect it directly; but it contains a substance which renders the phagocytic power of the leucocytes extremely active. Even in the presence of small quantities of this serum, the white corpuscles rapidly ingest the streptococci and are capable of stopping all development so long as they retain their amoeboid movements." "The action of the serum upon the leucocyte in its conflict with the streptococcus, is really derived from the horse immunised against this organism. It exists neither in the [329] ordinary horse nor in the horse vaccinated against diphtheria" (p. 15). Against these experiments of Denys and Marchand we might bring the same objection that we raised against the analogous experiments of Denys and Leclef, because, in both cases, these writers lay too much stress on the presence or absence of the phenomena of phagocytosis in preparations kept outside the body of the animal. Under these conditions phagocytosis is effected in a fashion too artificial to be capable of furnishing exact information.

Von Lingelsheim[2] met Denys and Marchand with the fact that, in their researches, the serum of the horse immunised against the streptococcus was only feebly bactericidal. After a prolonged contact (6—12 hours) with a specific serum, the streptococci, when transferred to rabbit's blood, showed retarded development as compared with streptococci subjected to the influence of the anti-diphtheritic and antitetanic horse serum. Von Lingelsheim himself, however, points out that the bactericidal action of the antistreptococcic serum was feeble and transient, and required the intervention of the reaction of the animal cells within the body.

The researches carried out by Bordet[3] in my laboratory are not open to the objections that we were justified in putting forward against the experiments made by Denys and Marchand, since he carefully watched the phenomena of immunity as they developed in the

[1] *Bull. Acad. roy. de méd. de Belg.*, Bruxelles, 1896.

[2] *Habilitations-Schrift*, Marburg, 1899, and in von Behring's "Beiträge zur experimentellen Therapie," 1899, Bd. I, S. 40.

[3] *Ann. de l'Inst. Pasteur*, Paris, 1897, t. XI, p. 177.

body of the animal subjected to the action of antistreptococcic horse serum. Bordet began by studying the properties of this serum and accepted Denys' and Marchand's statement that bactericidal power, however small, was absent. The streptococcus grows as well in this serum as it does in that of the untreated horse. In the specific serum, however, markedly longer chains are produced than in normal serum. This difference is found only in the earliest period of the growth. The agglutinative power of the antistreptococcic serum is but feebly marked. The injection of a large quantity of this serum into a normal rabbit confers no bactericidal power upon the serum of this animal. "The serum extracted 24 hours after injection is quite as suitable for use as a culture medium as that furnished by the blood before the introduction of the serum. In both the micro-organism grows rapidly and vigorously" (p. 195). Consequently, in
[330] the antistreptococcic serum there is nothing comparable to what we obtain so readily with antivibrionic serum: nothing which recalls Pfeiffer's phenomenon, even of an attenuated nature. We have already noted the result obtained by Bordet, according to which the streptococci, developed in the specific horse serum, were found to be endowed with their normal high virulence.

The antistreptococcic serum, injected into the peritoneal cavity of the rabbit the day previous to the microbial inoculation, protects the animal absolutely, provided that the micro-organisms be not too numerous or the quantity of serum not too small. Under these conditions the virus is ingested pretty rapidly and, so far as we know at present, completely. The micro-organism is thus prevented from developing and the animal remains in good health, whilst the control animal, which has received no serum, dies in a very short time.

When the number of streptococci is increased the effort of the animal organism to get rid of them becomes, in spite of the protective serum, more severe and much more prolonged. Some of the micro-organisms certainly become the prey of phagocytes, but a sufficient number remain free in the peritoneal cavity to multiply rapidly. When the number of streptococci has become sufficiently great a phenomenon, to which Bordet gives the name of "phagocytic crisis," is suddenly observed. In the peritoneal exudation, which has become thick and has taken on the aspect of a homogeneous and white pus, a most rapid phagocytosis is evidently set up. In a short time the whole of the streptococci, which were swarming outside the cells, are ingested by the leucocytes. "The essential condition for

recovery is always this complete ingestion" (p. 203). If the ingestion is not general, the rabbit may die, although much later than the control animal.

The phases of the struggle between the animal organism, when subjected to the influence of the protective serum, and the streptococcus, recall Salimbeni's experiments on immunised horses. The rabbit, in which phagocytosis could not take place at once owing to the presence of too large a number of micro-organisms, exhibits first a stage of free development of the streptococci, after which the phagocytes begin to fulfil their antibacterial function. Here it is especially the macrophages which act; the microphages, although present in fairly large numbers, are entirely inactive. This first stage of phagocytic reaction is insufficient. It is followed by a period when [331] the streptococcus appears to gain the upper hand. Many small chains, having escaped the phagocytes, multiply and give birth to quite a new generation of micro-organisms. If a fresh impulse to phagocytosis does not take place the animal dies from infection. When, however, the protective serum has been of sufficient strength, a new army of leucocytes arrives on the scene and these become masters of the situation. Phagocytosis becomes complete and microphages as well as macrophages devour a large number of streptococci.

Bordet, who, through his previous investigations, was well acquainted with the direct action of the protective serum on vibrios, could find nothing resembling it taking any part in the struggle of the organism of the animal treated with antistreptococcic serum against the streptococcus. The most that he could find was that the streptococci which again begin to swarm in the exudation are smaller in size than the normal streptococcus. It must be accepted, as indicated by the most recent researches, that this micro-organism becomes permeated by the fixative substance of the specific serum. We know already, however, that this fixation cannot deprive the micro-organisms of their virulence. In any case, then, a large share in the process must be attributed to the action of the phagocytes, stimulated by the protective serum, in the struggle of the animal against the streptococcus.

Having considered this series of examples of immunity against bacteria conferred by specific serums, we are in a position to form some idea of the mechanism of this immunity. Before we come to any general conclusion, it may be useful to glance at an example of this so-called passive immunity against a micro-organism belonging to

the animal kingdom. Such examples are not numerous, as, in the majority of cases of acquired immunity against Protozoan parasites, the serum is inactive and incapable of communicating immunity to normal individuals. We have only a single example, the *Trypanosoma* of rats, against which Dr Lydia Rabinowitch and Dr Kempner[1] have demonstrated the possibility of immunisation by the blood serum of vaccinated white rats. The mechanism of this immunity has been studied by Laveran and Mesnil[2], who found that it was like that described (Chap. VIII) in connection with the immunity in white rats, conferred by the inoculation of living *Trypanosomata*. The specific
[332] serum does not affect these infusoria except that it brings about slight agglutination. *Trypanosomata* placed in contact with it retain their pristine vitality and motility. This fact led Mme Rabinowitch and Dr Kempner to advance the hypothesis that the protective action of the serum must depend upon its antitoxic power. Since, however, in the infection of rats by the *Trypanosomata*, the toxic action is very feeble if not *nil*, it is very difficult to accept this view. It certainly appears to be much more probable that the serum acts in this case, as in many others, by stimulating the phagocytic reaction. The rapidity with which the living *Trypanosomata* are ingested by the phagocytes has been shown by Laveran and Mesnil.

Reviewing the whole of the data on immunity produced under the influence of anti-infective or protective serums, it is evident that they fall under two main categories. On the one hand there is a direct action of these serums on the micro-organisms, an action that is either microbicidal properly so called, agglutinative, or fixative. On the other hand, a stimulation of the phagocytic defence which leads to the final destruction of the micro-organisms is set up. This last factor is general; even in the case where the direct action is most marked (vibrios in the phagolysed peritoneal cavity), its importance is considerable. The micro-organisms which can be deeply injured by the direct action of the specific serum are few in number. In most cases this action is a feeble one and needs, for its completion, effective co-operation on the part of the phagocytes. In this respect micro-organisms present a whole gamut which begins with the cholera vibrio, the micro-organism most sensitive to the action of the body fluids, and ends with the *Trypanosoma* of the rat, a flagellated

[1] *Ztschr. f. Hyg.*, Leipzig, 1899, Bd. xxx, S. 251.

[2] Laveran, "Titres et travaux scientifiques," Paris, 1901, p. 39. *Ann. de l'Inst. Pasteur*, Paris, 1901, t. xv, p. 673.

Infusorian which cannot have even its motility affected by the direct action of the fluid elements. In all these cases, of course, the immunity conferred by the serums is due to the final destruction of the micro-organisms which invariably resolves itself into the same fundamental act—digestion by the cytases, a phenomenon which can only be produced at all quickly by the action of cytases contained in the protective serums or that have escaped from the phagocytes during phagolysis. The digestion by the cytases may also, and this is usually the case, be effected only after the manifestation of a regular series of vital phenomena on the part of the defensive elements of the body. As this factor fills such an important *rôle*, it is readily understood that we can not accept the term passive immunity by which to designate the immunity conferred by the specific serums. The action of the cytases, which is necessary to bring about the final [333] result in this immunity, depends too much on the activity of the cells which contain the bactericidal ferment. For this reason, when the functional activity of the phagocytes is in abeyance or is retarded, the animal succumbs, in spite of the presence in its organism of a more than sufficient quantity of cytases. In this connection Wassermann's[1] suggestion of adding normal serums rich in cytases to the specific serums must be regarded as very apposite. When protective serums poor in cytases or which have lost them as the result of heating, of the use of antiseptics, or simply from the influence of time, are injected, no immunising effect is ever obtained, simply because of the inactivity of the phagocytes, the cells in which the cytases are found. If at the same time normal serum rich in cytases ready prepared be injected, better results should be obtained. We may recall here an analogous example—the anthrax of the rat. Although possessing a large quantity of cytase, very effective against the bacillus, the organism of the rat can make no use of it, because the phagocytes which contain it do not manifest a sufficient activity. But the injection into a rat of blood serum from the same species containing a certain amount of cytase that has escaped during the formation of the clot, is sufficient to preserve the animal against a fatal infection.

To support his view, sound in principle, Wassermann made an experiment the interpretation of which presents certain difficulties. He injected guinea-pigs with protective antityphoid serum, in a dose insufficient to protect them against a fatal infection. By introducing along with this serum a certain quantity of normal ox serum which,

[1] *Deutsche med. Wchnschr.*, Leipzig, 1900, S. 285.

by itself, is also incapable of averting a fatal issue, Wassermann obtains an absolute immunity of his animals. This immunity is due, according to Wassermann, to the cytase of the ox serum acting along with the fixative of the specific serum. The united action of the two ferments causes the death of the micro-organisms. Besredka[1] has justly observed that normal ox serum contains, in addition to cytases, a substance which exerts a distinct agglutinative action on the typhoid cocco-bacillus and another which stimulates the phagocytic action. These two substances resist a temperature of 55°—60° C., and Besredka
[334] shows that with normal ox serum, deprived of its cytases by heating as above, we can obtain the same protective effect as with the same serum unheated.

As the result of another series of experiments, Wassermann[2] recognises the immunising action of normal serum heated to 60° C. and so entirely deprived of its cytases. Into the peritoneal cavity of guinea-pigs he injects, mixed with heated normal rabbit's serum, a dose of typhoid cocco-bacilli several times greater than the lethal dose. The guinea-pigs resist this completely. Analysing the mechanism of this immunity, Besredka (*l.c.* p. 229) attributes it to the combined action of the agglutinin and of the substance which stimulates the phagocytes. We have here another proof that the stimulins which play such an important part in immunity conferred by serums, are found not only in the specific serums, but also in normal serums, whether unheated or heated to 55°—60° C.

The protective property of the normal serums of man and animals against the cholera vibrio has already been referred to. We may now go a little more deeply into the mechanism by which these serums act. This task is an easy one thanks to the important work by Issaeff[3] carried out in R. Pfeiffer's laboratory. Having confirmed the observation, made by other investigators, that blood serum from the human subject, whether in health or affected by any disease, is capable of protecting the guinea-pig against the cholera vibrio provided that it is injected 24 hours before the micro-organisms, Issaeff studied the phenomena observed in the peritoneal cavity of the animals experimented upon. By means of small capillary pipettes he drew off at intervals a small quantity of fluid from the peritoneal cavity and examined it in hanging drop or in stained preparations. Some time

[1] *Ann. de l'Inst. Pasteur*, Paris, 1901, t. xv, p. 225.
[2] *Deutsche med. Wchnschr.*, 1901, S. 4.
[3] *Ztschr. f. Hyg.*, Leipzig, 1894, Bd. xvi, S. 287.

after the injection this fluid became more and more rich in leucocytes which seized the vibrios, ingested and destroyed them. To obtain this protective effect it was necessary to inject from 0·1 to 5 c.c. of human blood serum. With these doses he could prevent, not only infection of the guinea-pigs by the cholera vibrio, but also the lethal effects of other vibrios. The protective action of normal human serum is general, therefore, and not specific, such as is the immunity conferred by the serums of vaccinated animals or of the human subject who has suffered from an attack of cholera.

Shortly afterwards Funck[1] confirmed this result in the case of the [335] typhoid cocco-bacillus. He observed that normal horse's serum, injected as a protective agent in the dose of half a c.c. into the peritoneal cavity of the guinea-pig, preserved this animal from a fatal infection. Pfeiffer and Kolle and Chantemesse and Widal obtained the same results with human serum. The former observers lay special stress on the non-specific character of this protective action of normal serums. As to its mechanism, Funck sums it up as follows: "the specific serum brings about a rapid lysis of the bacilli, normal serum acts in a much more limited fashion; if the dose is very large and if the animal resists infection, the phenomena of extracellular degeneration are rarely appreciable, and it seems that here the specially important factor is the intracellular destruction of the bacteria, in the phagocytes" (p. 70).

Wassermann has shown the protective action of normal serum against the experimental disease produced by the staphylococcus. This action, although not absolutely general, is nevertheless widely distributed. Wassermann[2], from comparative investigations on this subject, came to the conclusion that "the serum of a different species of animal acts by greatly increasing the resistance, whilst the serum of the same species produces an effect which is not nearly so marked." As in these normal serums a stimulating influence on the phagocytes is specially marked, it may readily be understood that the serum of the same animal or of the same species does not produce so energetic an effect as the serum of a different species. As these normal serums possess, not only the property of exciting phagocytosis, but often also that of rendering motionless and of agglutinating certain micro-organisms, there might be some difficulty in interpreting the part played

[1] "La Sérothérapie de la fièvre typhoïde," Bruxelles, 1896, p. 69.
[2] *Ztschr. f. Hyg.*, Leipzig, 1901, Bd. XXXVII, S. 199.

by these serums. It may be useful, therefore, to pass in review the protective action of fluids less complicated than blood serums.

Issaeff, in the work already cited, demonstrated that not only normal serums but a whole series of fluids, such as urine, broth, etc., exert a protective effect against microbial infections. These fluids must be injected about 24 hours before the introduction of the bacteria. The best method consists in injecting them directly into
[336] the peritoneal cavity, after which the animals acquire an immunity against absolutely fatal doses of cholera vibrios. Funck verified this observation for the infection caused by the typhoid cocco-bacillus, and Bordet confirmed it for the streptococcus. The injection of peptonised broth into the peritoneal cavity of the normal guinea-pig, made 24 hours before an inoculation of double the fatal dose of the streptococcus, exerts a distinct protective action; the infection does not kill the animal. This broth is neither bactericidal, attenuating, nor agglutinative; it forms a good culture medium for the streptococcus and possesses no fixative power. Consequently it does not act directly on the vitality or virulence of the micro-organism; nevertheless, it is distinctly protective.

According to Issaeff's researches, the protective substances used by him must be arranged in the following order as regards their action against the cholera vibrio. Tuberculin is the most effective; then comes a 2% solution of nuclein, followed by normal human serum, broth, and urine, whilst physiological saline solution is the least active. All prevent infection by the vibrios, but the protection is effective for some days only; this protective action is exerted against various kinds of bacteria, being in no sense specific.

Pfeiffer lays so much stress on the great difference between the protective power of normal serums, as well as of the other fluids mentioned, and that of the anti-infective specific serums, that he even proposes to classify the first group as giving rise to *pseudo-immunity* or *resistance*. This view is certainly an exaggerated one, because it is difficult to draw a very distinct line between the two groups of phenomena. There are normal serums, of which 0·1 c.c. is quite sufficient to confer the protective effect, just as there are specific serums of which it is necessary to make use of a much greater dose to attain the same result.

Protective fluids, other than the serums, only manifest their influence by exciting a great phagocytic "superactivity." As the result of their injection into the peritoneal cavity of normal guinea-pigs,

first a transitory phagolysis is induced, this being soon replaced by a very considerable afflux of leucocytes, which is maintained for 24 hours or longer, and then gives place to the normal condition. It is during the period of the greatest leucocytosis of the peritoneal fluid that the animal exhibits the most marked resistance against infective micro-organisms. The vibrios are rapidly ingested by the phagocytes, without having previously been acted upon by the "humours." Bordet shows that the same thing happens in the case of the streptococcus inoculated into guinea-pigs after a protective injection of peptonised broth. [337]

We have observed the same phenomenon in guinea-pigs and white rats inoculated with the cocco-bacillus of plague. Treated with freshly prepared peptonised broth

Fig. 42. Culture of the plague bacillus developed within a macrophage from guinea-pig.

Fig. 43. Macrophage from guinea-pig filled with plague bacilli.

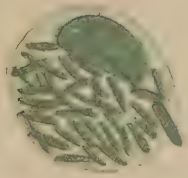

Fig. 44. Macrophage from guinea-pig containing plague bacilli which are commencing to escape from the protoplasm.

Fig. 45. Macrophage from guinea-pig which has burst as the result of the development of plague bacilli within it.

the day previous to inoculation, these animals oppose to the micro-organism a much more marked resistance than do the control animals. The injection of the cocco-bacillus of plague sets up a marked phago-

cytosis on the part of the macrophages. These cells ingest large numbers of micro-organisms which, after a time, have all passed into the phagocytes. If a drop of the peritoneal exudation is now with-
[338] drawn, we find only intracellular cocco-bacilli (fig. 43). If the drop be kept for some time outside the animal and at a suitable temperature the macrophages may be seen to perish and the micro-organisms to develop in their contents. We thus obtain abundant cultures which pass from the interior of the macrophages into the fluid of the exudation (figs. 42, 44, 45). When the animals are not sufficiently protected the same phenomenon is observed in the peritoneal cavity of the living animal. The macrophages, crammed with cocco-bacilli, burst, allowing the micro-organisms to escape. These multiply in the peritoneal fluid and spread through the animal, which soon dies.

Wassermann affirms that "the artificially increased resistance is nothing but an active and reinforced afflux of the complements (cytases) towards one point in the animal, for the purpose of digestion." (*Ztschr. f. Hyg.*, Leipzig, 1901, Bd. XXXVII, S. 199.) Wassermann does not explain how this afflux of cytases is produced. The entirely concordant researches on this point by Issaeff, Funck, Bordet, and ourselves, prove that this afflux takes place not through the mediation of the fluids, but solely through the phagocytes, the carriers of the cytases. Consequently it is beyond dispute that in the immunity conferred by physiological saline solution, broth, and several other fluids, we have to do solely with an augmentation of the phagocytic reaction. In the immunity conferred by normal or specific serums, this same stimulating factor still plays the more important part. Along with it, however, there is an intervention more or less pronounced, according to circumstance, and more or less frequent, of cytases, brought by the serums prepared outside the body or that have escaped during phagolysis, as well as of substances truly humoral, such as the fixatives or the agglutinins.

Amongst the non-specific substances which are capable of conferring an immunity more or less stable, must be placed the products of micro-organisms other than those against which we wish to protect the animal. Pasteur[1] noted that when the anthrax bacillus, mixed with other micro-organisms, in themselves inoffensive, is inoculated into animals, anthrax does not develop and the animals remain well. Later, Emmerich[2] showed that the streptococcus of

[1] *Compt. rend. Acad. d. sc.*, Paris, 1877, t. LXXXV, p. 107.
[2] *Arch. f. Hyg.*, München u. Leipzig, 1887, Bd. VI, S. 442.

erysipelas exerts an antagonistic influence against the anthrax bacillus. He succeeded in immunising and even in curing rabbits [339] inoculated with anthrax, by submitting them to the action of this streptococcus.

These experiments served as the starting-point for several works on the vaccination of animals against anthrax by means of various micro-organisms, as well as by their products. Pawlowsky[1], Watson-Cheyne[2], and Bouchard[3] have proved that bacteria not very pathogenic and even saprophytes, such as the *Cocco-bacillus prodigiosus*, Friedländer's bacillus, and the *Bacillus pyocyaneus*, were also capable of preventing infection by the anthrax bacillus. Freudenreich[4] showed that not only did the bacillus of blue pus exert an antagonistic action but that the same effect could be obtained with sterilised cultures of this organism. Woodhead and Cartwright Wood[5] studied the vaccinating action of these products on rabbits inoculated with virulent anthrax bacilli. The animals resisted completely or survived for some time. Analysing the phenomena produced under such conditions, these two authors came to the conclusion that the action of sterilised cultures of *Bacillus pyocyaneus* is "indirect and as taking place either by opposing itself to the action of the poison upon the tissues, or by stimulating certain tissues and increasing their functional activity." With the object of obtaining an exact interpretation of this antagonistic influence I suggested to M. Blagovestchensky[6] that he should investigate in detail the phenomena which take place in the organism of rabbits inoculated with the anthrax bacillus and submitted to the action of sterilised cultures of the *Bacillus pyocyaneus*. At the very outset this observer was met by the fact that these cultures act directly upon the vitality of the anthrax bacillus. Thus the association of the former with the anthrax bacillus *in vitro* was sufficient to interfere with the development of the latter. Under these conditions he had to renounce the investigation of the part played by the cellular elements of the rabbit in the antagonism of the two bacteria.

Friedländer's bacillus has been found to be much more suitable for this line of research as is shown by work carried out by Freiherr

[1] *Virchow's Archiv*, Berlin, 1887, Bd. CVIII, S. 494.
[2] *London Medical Record*, 1887.
[3] *Compt. rend. Acad. d. sc.*, Paris, 1889, t. CVIII, p. 713.
[4] *Ann. d. Microgr.*, Paris, 1889, p. 465.
[5] *Compt. rend. Acad. d. sc.*, Paris, 1889, t. CIX, p. 985.
[6] *Ann. de l'Inst. Pasteur*, Paris, 1890, t. IV, p. 689.

[340] von Dungern[1] in my laboratory. This observer convinced himself that "anthrax bacilli are weakened neither by the encapsuled bacilli nor by the substances which they contain." These micro-organisms do not interfere in the slightest with the anthrax bacilli either outside or within the animal, and "when the anthrax infection does not become generalised it is due to the fact that the anthrax bacilli are ingested by the phagocytes at the seat of inoculation and destroyed within these cells" (p. 183).

In this action of foreign micro-organisms upon micro-organisms against which we wish to protect the animal we have to deal with something analogous to the condition we obtain when immunising with normal serums or with any other kind of fluid. In both cases immunity is rapidly established, but it is very transient and is confined to a stimulation of the phagocytic resistance. Direct action may also intervene, as in the case of *Bacillus pyocyaneus*, but it is not indispensable. The animal whose phagocytes are in a condition of superactivity can do without this direct action, its own resources being sufficient to arrest anthrax.

Following the same lines of investigation as those on the antagonism between the anthrax bacillus and several other micro-organisms, Klein[2] has demonstrated that, in order to prevent a guinea-pig from contracting experimental cholera peritonitis, it is only necessary to inject into it, the day before infection, a culture of Finkler and Prior's vibrio or of certain other bacteria. These experiments by Klein served as the point of departure for Issaeff's work which led to the discovery of the stimulating influence of all kinds of fluids injected into the peritoneal cavity of guinea-pigs.

In this transient immunity obtained with products foreign to the micro-organism against which one is vaccinating, the most constant and consequently most important part is again played by the phagocytes. But there is associated with it an influence, greater or less in degree, of substances present in the serums, such as the microcytases and fixatives, which are able to exercise a direct action on the pathogenic micro-organisms. In all cases known and analysed up to the present, the intervention of the living organism of the animal is indispensable, consequently this form of acquired immunity against micro-organisms cannot be regarded as being really passive.

[1] *Ztschr. f. Hyg.*, Leipzig, 1894, Bd. XVIII, S. 177.
[2] *Centralbl. f. Bakteriol. u. Parasitenk.*, Jena, 1893, Bd. XIII, S. 426.

CHAPTER XI [341]

NATURAL IMMUNITY AGAINST TOXINS

Examples of natural immunity against toxins.—Immunity of spiders and scorpions against tetanus toxin.—Immunity of the scorpion against its own poison.—Antivenomous property of the blood of the scorpion.—Immunity against tetanus toxin in the larvae of *Oryctes* and in the cricket.—Immunity and susceptibility of frogs against this toxin.—Natural immunity of reptiles against tetanus toxin.—Antitetanic property of the blood of alligators.—Immunity of snakes against snake venom.—Immunity of the fowl against tetanus toxin.—Immunity of the hedgehog against poisons and venoms.—Immunity of the rat against diphtheria toxin.

As in this book we are dealing specially with the immunity against infective diseases, the question of the resistance of the animal to poisons interests us only in so far as it is related to immunity against micro-organisms. Consequently the reader must not expect a treatise on intoxications properly so called nor one on immunity against all kinds of poisons. To perform such a task we should have to far overstep the bounds of the subject that we have chosen and enter upon an examination of questions which are beyond our sphere. Our chief aim is to present to the reader a summary of our present knowledge on immunity against microbial toxins and to establish the relations between this kind of immunity and immunity against infective micro-organisms. In order to do this, however, we shall have now and again to go beyond the limits of our programme and discuss certain problems bearing on the resistance of the animal organism against poisons not of microbial origin.

The immunity against toxins, like that against the micro-organisms themselves, may be either natural or acquired. As many poisons have been known from time immemorial, we are able to collect numerous observations on the resistance of the animal organism to such substances made when there was no idea of immunity against infective diseases. The etiology of intoxications is often much more evident and simple than is that of infections; this is one of the [342]

reasons that the older conceptions on the subject of immunity against poisons were more advanced than were those on immunity against infective diseases.

Several examples of natural immunity in the lower animals have already been cited. Thus, we have seen in previous chapters that the Infusoria are resistant to poisons that exert a powerful action on a large number of the higher animals, such as the tetanus and diphtheria toxins and especially the ichthyotoxin of eel's serum. We have mentioned the case of the larva of *Oryctes nasicornis* which is unaffected by large doses of the toxins of certain bacteria and which at the same time is very subject to fatal infections by very small doses of the bacteria that form the poisons. These larvae, like those of the cockchafer, are, however, fairly susceptible to the poison of the scorpion. Several other species of Arthropoda, which have been studied from the point of view of immunity against toxins, have exhibited analogous features. Thus spiders and scorpions are refractory to tetanus toxin. In one experiment I injected into the abdominal cavity of a *Mygale* from the Congo (which weighed 7 grm. 5) 1 c.c. of tetanus toxin on two several occasions. This dose is sufficient to kill, with the symptoms of tetanus, 1000 mice of double the weight. The spider, kept in the incubator at 36° C., remained quite well during the two months that the experiment lasted. It exhibited no symptom, not even transient, of muscular stiffening, nor any change in its habits and natural functions. The tetanus toxin disappeared from the blood of the *Mygale*, but this blood at no time showed the slightest antitoxic power against this poison. This example of natural immunity cannot, therefore, be ascribed to any antitoxic property of the fluids and must be regarded as a case of immunity of the tissues—von Behring's histogenic immunity. In the present imperfect state of our knowledge it is impossible to describe precisely the mechanism of this immunity. When we say that the spider is refractory to the tetanus toxin because its susceptible elements have no receptors capable of seizing the haptophore group of this poison, we simply give expression to a hypothesis which we are not in a position to verify by experiment.

The scorpion, a well-known representative of the Arachnida with
[343] segmented abdomen, shares with the *Mygale* in the immunity against tetanus toxin. The Algerian and Tunisian scorpions (*Scorpio afer* and *Androctonus occitanus*) withstand the action of doses of this poison which are fatal for 1000 mice and more. Taking weight as our

standard we may inject into them, with impunity, more than 5000 times as much toxin as into mice, without setting up a single morbid symptom. Scorpions, like the *Mygale*, live well in the incubator at 36° C., where they are kept whilst submitted to the action of the tetanus poison. Here again we have to do with a case of histogenic immunity. The fluids of the scorpion exert no antitoxic action. When blood from the normal scorpion is mixed with various doses of tetanus toxin and injected into mice these animals contract tetanus and die just as do the control animals. In certain exceptional cases some slight retardation was observed, but the blood of the scorpion is, in most cases, incapable of preventing tetanus in animals susceptible to this disease.

Scorpions, injected with tetanus toxin, do not retain it in their blood for long. A few days after the injection of the tetanus poison such blood, when injected subcutaneously into mice, excites no trace of tetanus. The preparation of extracts of the different organs of scorpions treated with tetanus toxin demonstrates that the liver and the liver only absorbs the poison. It is found there a few days after the injection of the toxin and it remains there unaltered for some considerable time. The exudation of the liver of scorpions, killed a month or more after the introduction of the toxin into the general cavity, injected into mice sets up a typical and fatal tetanus.

The presence of the tetanus toxin in the organism of scorpions does not give rise to the production of antitoxin. At any rate a whole series of experiments on this point carried out by us never gave a positive result. The scorpions resisted repeated doses of the tetanus toxin and lived without any difficulty at 36° C., but their blood was never at any period capable of preventing mice from contracting fatal tetanus. Nevertheless the scorpion may possess antitoxic power.

Everyone has heard of the supposed suicide of the scorpion. We are told that when this animal finds itself under conditions in which its death is inevitable, it stings itself with the end of its tail and dies from the effect of its own poison. A simple method of reproducing [344] this experiment is actually described:—Surround the scorpion with a circle of fire. The animal rushes in all directions to find a way out, and finding none, deliberately commits suicide. Bourne[1] at Madras carefully investigated this question in a large species of Indian

[1] *Proc. Roy. Soc. London*, 1887, Vol. XLII, p. 17.

scorpion and demonstrated the absolute erroneousness of the story of suicide which, had it been true, would have afforded a unique example of voluntary death in animals. On carrying out the classic experiment he observed that within this ring of fire the scorpion is subjected to a very high temperature. When the temperature reaches 40° C. the scorpion begins to grow weak and as the temperature approaches 50° C. it passes into a comatose condition. Moreover Bourne showed that the scorpion's poison, which is fatal for large spiders, insects, and vertebrates, was innocuous for individuals of the species furnishing it.

I can confirm all the statements of this English observer. When I was studying the embryology of the scorpion I repeatedly tried the experiment but the animal never committed suicide. Further, I repeatedly assured myself of the innocuousness of the scorpion's poison when injected into individuals of the same species, and I was able to demonstrate most conclusively that the blood of the scorpion is endowed with undoubted antitoxic power. The addition of 0·1 c.c. of this blood to a dose of poison which kills mice in half-an-hour is sufficient to enable a mouse injected with the mixture to resist it completely. This antitoxic power is the same in the *Scorpio afer* and in the Algerian *Androctonus*. An emulsion of the liver of the scorpion, however, is absolutely incapable of preventing fatal intoxication of mice.

This case of antitoxic action is the only one I have been able to demonstrate in an invertebrate. Must we regard it as a case of natural innate antivenomous power or as something acquired during the life of the animal? It is not easy to settle this question by experiment. We can certainly procure new-born scorpions and rear them for some time, but the quantity of blood that can be got from them is insufficient for injection for protective purposes. Scorpions do not love one another and when kept together we often find them engaged in fierce and mortal combat, the stronger killing the weaker and sucking their blood. It is therefore possible that, in some stage of their life, the scorpions find means of vaccinating themselves against their own poison either through the intestine or as the result
[345] of punctures caused by the point of the tail. It would be very interesting to study this question under favourable conditions, because it is capable of throwing light on the problem of the origin of antitoxins, from a general point of view. Whichever view be taken, the acquisition of any antitoxic property by the blood in the Invertebrata

must take place slowly and with great difficulty as is shown by our want of success with tetanus toxin.

Insects are, as a rule, very tolerant of this latter poison. As, however, the tetanus toxin (we shall illustrate this later) only acts well and in small doses at a high temperature (about 30° C.) and as most insects do not readily adapt themselves to this temperature, it was necessary to choose species capable of living at these high temperatures and for this line of study the larva of *Oryctes* is most suited. It flourishes well at a temperature of 30°—36° C., and under these conditions exhibits a much greater resistance to infection by *Isaria* than at lower temperatures. It can be kept in the incubator for months if placed in glass jars filled with earth mixed with tanner's bark. The injection of enormous quantities of very active tetanus toxin directly into the blood has not the slightest effect on these larvae. Whilst, however, the blood fluid of the Arachnida rapidly gets rid of the poison, that of *Oryctes* retains it for a very long period. If a small quantity of blood be taken from larvae several months after injection and then injected into mice, these animals contract typical tetanus and quickly succumb.

The toxin, however, finally disappears from the blood though a certain portion of it may still be found in the pericardial cells and especially in the fat-bodies.

Never, under any circumstances, was I able to observe that the blood of the larvae of *Oryctes* exerted any antitoxic action. At the stage when this fluid no longer gives tetanus to mice, it is absolutely incapable of preventing intoxication when mixed, before injection, with tetanus toxin.

Amongst adult insects the cricket is best adapted for researches on tetanus. The field cricket will bear a temperature even higher than 30° C. It is completely resistant to injections of tetanus toxin, but it showed no more capacity than did the larvae of *Oryctes* or the Arachnida of producing any tetanus antitoxin.

All the Invertebrata that I have been able to study have exhibited a remarkable resistance against the known bacterial toxins, but the [346] mechanism of this natural immunity could not be exactly made out owing to the difficulty met with in investigating the toxins in the organs and following their modifications. The idea of making use of these lower animals for the purpose of solving the problem of the origin of antitoxins is not realisable, from the fact that the Invertebrata that have been studied have never, in my experience, produced

any of these substances as the result of injections, whether single or repeated, of toxins.

The natural immunity of the Invertebrata against bacterial toxins cannot therefore be regarded as an example of humoral immunity. It must be placed in the category of histogenic immunity, although we are not in a position to define accurately the part played by the cellular elements in the defence of the animal against these poisons. We must, therefore, go higher up in the animal scale if we are to solve the principal questions in regard to antitoxic immunity.

The lowest Vertebrata, the fishes, are not well-suited for this kind of research. The best known bacterial toxins act specially on warm-blooded animals and require the co-operation of high temperatures. Fishes do not live well in captivity except at relatively low temperatures and soon die if placed in an incubator kept at 30° C. or higher. It is necessary, therefore, to have recourse to the Amphibia, which are much more easily acclimatised to these temperatures. The Axolotl, coming from Mexico, is naturally capable of withstanding great heat. These animals will live for long at a temperature of 30°—37° C. They possess the drawback, however, of being very susceptible to the tetanus toxin, very small doses of it being fatal. The green frog (*Rana esculenta*) is the most suitable for our purpose. It readily adapts itself to optimum temperatures (30°—36° C.) and exhibits at least a certain degree of immunity against various bacterial toxins. We have stated in a preceding chapter that the green frog is unaffected by considerable quantities of diphtheria toxin. It is resistant also to tetanus toxin, but this natural immunity appears to be connected with special conditions. Courmont and Doyon[1] were
[347] the first to draw attention to the fact that beyond 20°—25° C. green frogs may contract tetanus. Refractory in winter they become susceptible in summer. These observers afterwards found that of frogs inoculated with the same dose of toxin and divided into two sets, one set kept at a temperature of about 10° C. remained quite well whilst the other set subjected to one of 30°—39° C. contracted tetanus after five days' incubation. This experiment has been confirmed by several observers, and indicates that the tetanus poison demands, for the manifestation of its toxic action, a favourable and fairly high temperature. This result must, however, be accepted with some reserve. Undoubtedly the doses of tetanus toxin which induce fatal tetanus in

[1] *Compt. rend. Soc. de biol.*, Paris, 1893, pp. 294, 618; 1898, p. 344. "Le tétanos," Paris, 1899, p. 25.

frogs kept at a high temperature are innocuous when these animals are living at low temperatures. But we can, by increasing the dose, produce tetanus in frogs even when the temperature is not very high. Thus Marie[1] was able, during the whole of the winter, to tetanise both green and brown frogs living in water the temperature of which oscillated between 13° and 18° C. The incubation period in this case is very much longer (sometimes extending to 25 days) than in frogs kept at higher temperatures.

Temperature, therefore, is an important factor in the poisoning by the tetanus toxin and in the resistance of the frog, but, in the long run, this poison can exert its specific action even at relatively low temperatures.

Morgenroth[2] endeavoured to analyse the mechanism of this resistance and of the susceptibility of the green frog when maintained at various temperatures. He demonstrated that the tetanus toxin is fixed in the central nervous system, even at low temperatures, near 8° C.; under these conditions, however, it is incapable of causing the slightest tetanic symptom. When placed in an incubator kept at 32° C. the frogs contract tetanus after a period of incubation of some (2 to 3) days. During the first 24 hours of this period the frogs manifest no sign of tetanus, and if they are again put in a cool place they continue in good health. If, however, after a not too prolonged stay in the cold, these animals are subjected a second time to [348]
the higher temperature, they become tetanic, after a shortened incubation period. Cold, therefore, may arrest tetanus even at a stage when the toxin has already produced certain latent but permanent modifications of the nervous system.

Frogs injected with tetanus toxin and kept in a cold place finally get rid of the poison. When transferred to a warm chamber after a certain lapse of time they no longer contract tetanus. We have found that the greater part of the tetanus toxin continues for some time in the blood of frogs injected and kept at a low temperature. A small quantity of this blood withdrawn almost two months after the last injection produced fatal tetanus in a mouse. We do not know how frogs eliminate the toxin, but it has been demonstrated that in this case it causes no production of antitoxin. Morgenroth has confirmed this result.

Reptiles must be regarded as vertebrates exhibiting a most

[1] *Ann. de l'Inst. Pasteur*, Paris, 1897, t. XI, p. 597.

[2] *Arch. internat. de Pharmacodyn.*, Gand et Paris, 1900, Vol. VII, p. 265.

pronounced natural immunity against tetanus. They show an unlimited resistance to enormous doses of tetanus poison, and this at low, medium, or high temperatures (30°—37° C.). Green lizards withstand considerable doses of tetanus toxin. Although they do not contract tetanus, they get rid of the poison exceedingly slowly. Thus, a lizard kept at a temperature of 20° C., and injected with an amount of toxin sufficient to kill 500 mice, at the end of two months still retains in its blood such an amount of the poison that one-tenth of a c.c. will cause fatal tetanus in a mouse. Turtles present an analogous case. The marsh turtle, *Emys orbicularis*, tolerates very large amounts of tetanus toxin, injected subcutaneously, and this at both low and high temperatures, at 30° C. and beyond (36°—37° C.). The toxin passes quickly into the blood and remains localised there for a very long time. In a turtle kept in an aquarium at the laboratory the blood was tetanigenic for the mouse even four months after an intra-peritoneal injection of the toxin. In another turtle which lived at incubator temperature (36°—37° C.), the blood was still toxic two months after a subcutaneous injection of tetanus toxin in quantity fatal for 500 mice. In turtles kept at 36° C. I observed abundant transudations into the peritoneal cavity, and the fluid, very poor in
[349] formed elements, was found to be very tetanigenic. It must be accepted, therefore, that the toxin is retained in the blood plasma with which it passes into the transudation. Every kind of cell must exhibit a very marked negative chemiotaxis against tetanus toxin for this poison to be retained so long in the body fluids. Under these conditions it is not surprising that in turtles I was never able to observe the slightest antitoxic power in the blood. Their great natural immunity must be due to some other factor.

The alligator (*Alligator mississippiensis*) has also been found to be quite refractory to tetanus both at low and at high temperatures. Outwardly alligators behave exactly as do turtles, that is to say, after the injection of various and sometimes very large doses of toxin they exhibit no morbid symptom either general or tetanic. But the particular changes which occur in their organism differ essentially from those met with in the turtle. The toxin is rapidly eliminated from the blood of the alligator, even when it is kept at a relatively low temperature (20° C.). Under these conditions of temperature, however, the blood does not become antitoxic although it has lost its tetanigenic property. When, however, the alligators are kept at a higher temperature (32°—37° C.), antitoxic power is developed in

their blood, often with very great rapidity. Quite young alligators (weighing about 500 grammes) are capable of producing antitoxin, though somewhat slowly. A month after the first injection of the tetanus toxin their blood is incapable of causing tetanus in mice, but is not yet antitoxic. A month later, however, it never fails to prevent an attack of tetanus when mixed with fatal doses of the toxin and injected into mice.

Older alligators develop antitoxic power much more rapidly, and on several occasions we have found, to our great astonishment, that, as early as 24 hours after injection of the toxin, their blood was distinctly antitetanic. The blood of the same alligators, tested before the injection of the toxin, like the blood of normal alligators generally, exhibited no antitoxic property.

In several experiments we took the rectal temperature of our animals and were never able to observe the slightest rise corresponding to the temperature of the water in which the alligators lived.

It cannot be doubted then, that, in spite of the facility with which [350] these reptiles produce tetanus antitoxin, their immunity does not depend on this antitoxic property. Thus, young alligators which have resisted a single dose of toxin sufficient to kill 6000 mice must owe their immunity to some other cause than the antitoxic power of the body fluids, for their blood does not begin to exhibit this property until two months after injection.

These same reptiles are also very refractory against cholera toxin, even in large doses; they react to the injection by the development of the corresponding antitoxin. On the other hand they are very susceptible to diphtheria toxin, small quantities of which are quite sufficient to bring about a fatal intoxication.

Snakes, like other reptiles, are refractory against tetanus toxin. In the study of their natural immunity, however, we are confronted by the difficulty that their blood is naturally toxic for laboratory animals. This toxin, analogous to the ichthyotoxin of eel's serum, has been compared with snake venom against which the snakes themselves enjoy a very marked immunity.

Not venomous snakes only exhibit immunity against their own poison. Long ago Fontana[1] observed that non-venomous snakes resist the bite of the viper and even subcutaneous inoculation of its venom. Phisalix and Bertrand[2] confirmed these observations

[1] "Traité sur le venin de la vipère," Florence, 1781.

[2] *Arch. de physiol. norm. et path.*, Paris, Année XXVI, 1894, p. 423.

and were able to show that a non-venomous snake (*Tropidonotus*) will withstand a dose of venom capable of killing from 15 to 20 guinea-pigs. Seeking for the cause of this natural immunity, these observers came to the conclusion that it is due to the presence in the blood of toxic substances analogous to those of the venom of the viper. These same substances are found also in the labial glands of the upper jaw of the *Tropidonotus* and can from thence, according to the view of Phisalix and Bertrand, pass into the blood as an internal secretion. Calmette[1] has shown that the blood of snakes, injected in a non-toxic dose, vaccinates certain mammals against snake venom, and Phisalix and Bertrand have even obtained an antitoxic effect by injecting a mixture of snake's blood, heated to 58° C., with lethal doses of venom. There is, then, in this example
[351] something analogous to what we have described in scorpions, with this difference, however, that the blood of these Arachnids is already antitoxic, to a certain degree, whilst that of snakes only becomes so after it has been modified by heat.

The classic example of immunity against a bacterial toxin amongst Birds is that of the fowl, which is highly refractory against the tetanus toxin. In the very earliest researches on this poison injections were made into vertebrates of very different kinds, and a very striking feature was the facility with which fowls resist very large quantities of tetanus toxin. However, as is almost always the case, this immunity has been found not to be absolute. By means of enormous doses, injected subcutaneously or into the muscular tissue, tetanus of the most typical kind, ending in death, has been induced in fowls, and in fowls weakened by cold, tetanic intoxication, even with smaller doses, has been set up. By injecting the toxin directly into the brain, according to Roux and Borrel's method, the fowl may be still more easily tetanised. Thus, von Behring[2] observed that by injecting one milligramme of the toxin into the brain of a fowl, weighing one kilo, tetanus may infallibly be produced.

After the brilliant and fruitful discovery of the antitoxic property of the blood, made by von Behring in collaboration with Kitasato, we were justified in concluding that immunity against toxins and, amongst others, natural immunity, might depend on the power of the body fluids to neutralise the toxins. This hypothesis has been formulated at various times, but it was for the first time subjected to

[1] "Le venin des serpents," Paris, 1896, p. 40.

[2] "Allgemeine Therapie der Infectionskrankheiten," Berlin u. Wien, 1899, S. 992.

experimental control by Vaillard[1], and specially in connection with tetanus in the fowl. The blood or blood serum of these birds, when mixed in varying doses, small, medium, and large, with tetanus toxin, was never found to be capable of preventing susceptible animals (mice, guinea-pigs, rabbits) from contracting tetanus: these animals so treated behaved just as did the controls inoculated with toxin only.

The great resistance of the fowl against tetanus,—one of the most typical examples of natural immunity against a microbial poison,—cannot, therefore, be explained by the presence in the body fluids of an antitoxin capable of neutralising and rendering innocuous the [352] tetanus toxin. On the other hand, we are not justified in attributing it simply to the absence of corresponding receptors in the sensitive nerve cells. Since the fowl readily contracts tetanus when the toxin is injected directly into the brain or when the fowl is weakened by cold, it is evident that the sensitive elements never fail to absorb and fix any poison that is presented to them. In ordinary cases, however, when the fowl exhibits its remarkable resisting power against the toxin injected in very large quantity, subcutaneously, into the muscles or into the peritoneal cavity, the poison does not reach the sensitive cells, being arrested and rendered innocuous whilst circulating in the tissues of the organism.

Von Behring[2] is of opinion that in examples of natural immunity, such as the one just examined, the principal cause of the refractory condition depends upon the impermeability to the toxin of the capillary wall of the vessels. It is, however, difficult to maintain this thesis in regard to tetanus in the fowl, when it is remembered how readily tetanus toxin passes through filters and membranes, and especially in view of the fact that weakening of the fowl by means of cold renders it susceptible to doses of toxin which are tolerated without inconvenience by normal fowls.

We are, therefore, compelled to place the natural immunity of the fowl against tetanus toxin in the category of cell immunities. This toxin, as we have said, must be arrested *en route* before it reaches the cells of the nerve centres. But where and how does this beneficent arrest take place? Ten years ago Vaillard demonstrated that the

[1] *Compt. rend. Soc. de biol.*, Paris, 1891, p. 462; *Ann. de l'Inst. Pasteur*, Paris, 1892, t. VI, p. 229.

[2] Article: *Infectionsschutz und Immunität* in Eulenburg's "Real-encyclopädie d. ges. Heilkunde" (Encyclop. Jahrbücher), Wien, 1900, Bd. IX, S. 203.

blood of fowls that have received an injection of tetanus toxin causes typical tetanus in susceptible animals. This tetanigenic property of the blood persists for a certain number of days. When it is measured by the quantitative method, it is found that all or almost all the tetanus toxin injected into the peritoneal cavity of the fowl passes into the blood and remains there intact for a variable number of days. From a morphological point of view the blood, immediately after the injection of the toxin, exhibits a hyperleucocytosis of greater or less duration.

When the fowls are killed at the stage when their blood becomes tetanigenic (as the result of the injection of the toxin into the peri-
[353] toneal cavity), it can be demonstrated that their viscera are not capable of producing tetanus in susceptible animals except in so far as they contain blood. It is only the vascular organs, rich in blood, such as the spleen, liver, kidneys, thyroid gland and bone-marrow, that impart tetanus and then only in so far as they have not been freed from blood. Of the various organs only the genital glands, ovaries or testes, absorb a certain amount of the injected toxin. Very young testes or the smallest ovarian ova containing as yet no trace of yellow yolk, when injected into mice, produce a fatal tetanus.

In fowls, insusceptible to tetanus toxin, this toxin is found, then, in the sexual glands and in the blood. When, in order to ascertain the exact localisation of this toxin, we measure the tetanigenic power of the whole blood as compared with that of the aseptic exudations induced by the injection of gluten-casein, and necessarily much richer in leucocytes, we get the result that the exudations contain more tetanus toxin than does the blood. We are led, therefore, to the conclusion that this poison is absorbed, at least in part, by the leucocytes, and it is in these elements and in the genital cells that we must look for the factors which arrest the toxin and prevent its reaching the nerve centres.

Cellular or histogenic immunity is often contrasted with chemical immunity without taking into consideration the real analogies and differences to be found between them. It is evident that in both groups the organism of the animal modifies the introduced toxins and that this modification is a chemical process. In cellular immunity, however, this act is preceded by certain biological phenomena, such as the reaction of the formed elements and the absorption of the noxious substance. Immunity in these cases is more complex than in the example where the toxin is neutralised by a direct action of the

body fluids, but ultimately it always resolves itself into a chemical or perhaps physico-chemical action of the substances of the organism of the animal on the toxic substances of the poisons.

In Mammals examples of natural immunity against certain poisons are not rare. Almost a century ago Oken made the observation that a person who tried to poison a hedgehog with opium, hydrocyanic acid, arsenic or mercury bichloride usually failed in his attempts because of the great resisting power of this animal. Harnack demonstrated that the hedgehog will withstand a dose of potassium cyanide six times as great as that necessary to kill a cat in a few [354] minutes (0·01 grm.). In Lewin's[1] experiments the hedgehog was found to resist the injection of powdered cantharides in a quantity seven times as great as that which infallibly kills a dog and greater also than the lethal dose for man. The same observer also confirms the observation that a much larger dose of alcohol must be used in order to intoxicate a hedgehog than is required to obtain the same effect in the rabbit or even in the dog. Horvath[2] fed hedgehogs for a fairly long period with living cantharides. These Insectivora devour their venomous prey without showing any sign of illness except a certain degree of emaciation. When Lewin tried to ascertain the cause of this natural immunity of the hedgehog he examined the blood of this animal for a substance antitoxic to cantharidine. His experiments were all negative; but it is difficult to come to any definite conclusion in this matter from the fact that the blood and blood serum of the normal hedgehog are toxic for the small laboratory animals. A similar objection had already been brought forward by Phisalix and Bertrand in connection with their experiments, analogous to those of Lewin, on the immunity of the hedgehog against the venom of the viper.

It has long been known that hedgehogs have a liking for certain reptiles and wage an implacable war on snakes in general and on the viper in particular. In its attack the hedgehog tries to avoid being bitten, but when, as often happens, it fails to evade a bite the inoculation of the viper's venom appears to be well borne. This observation has been confirmed experimentally. Phisalix and Bertrand[3] have shown that the resistance of the hedgehog to the

[1] *Deutsche med. Wchnschr.*, Leipzig, 1898, S. 373.

[2] *Vrach*, St Petersburg, 1897, p. 964.

[3] *Compt. rend. Soc. de biol.*, Paris, 1899, p. 77; *Bull. Muséum d. hist. nat.*, Paris, 1895, t. I, p. 294.

viper's venom is about forty times as great as that of the guinea-pig, that is to say the hedgehog, though far from possessing an absolute immunity, nevertheless exhibits a much greater resistance than do most animals. Lewin[1] convinced himself of this fact as regards adult hedgehogs, though young animals, according to him, are much
[355] more susceptible. Thus, he has seen a young hedgehog that had been bitten by a viper die after nine days' illness. This observation speaks in favour of the conclusion that the immunity of the hedgehog might be naturally acquired rather than a really natural immunity. The hedgehog, hunting all kinds of small animals, might often be bitten by vipers and in this way acquire its immunity against the venom. Under these conditions we can readily conceive that the blood of this "insectivoran" might be placed in a position to develop a specific antitoxic property.

When Lewin tried to satisfy himself of the existence of this property by direct experiment he could only show that the blood of the hedgehog was powerless to prevent the lethal effect of the viper's venom on small animals. But here, as in his researches on cantharidine, he did not take into account the inherent toxicity of the blood of the hedgehog. Phisalix and Bertrand[2], who have also studied this question, have obtained results at variance with those of Lewin. They demonstrated first of all that the blood of normal hedgehogs was capable of intoxicating and even of killing laboratory animals such as the guinea-pig. It is quite natural, therefore, that the mixture of this fluid with viper's venom could not be tolerated. It was, however, sufficient to heat the blood of the hedgehog to 58° C. for it to become not only innocuous of itself, but even for it to exhibit an antitoxic action against snake venom. Thus, guinea-pigs which had received 8 c.c. of heated hedgehog's serum into the peritoneal cavity, were at once in a condition to resist double the lethal dose of viper's venom. Phisalix and Bertrand conclude, therefore, that "the natural immunity of the hedgehog against the viper's venom is due to the presence in its blood of an immunising substance." The same observers[3] satisfied themselves that horse's serum and even that of the guinea-pig exercise an undoubted anti-venomous action; yet these animals are anything but insusceptible to snake venom. Moreover, the necessity to heat the blood to 58° C., as a

[1] *Deutsche med. Wchnschr.*, Leipzig, 1898, S. 629.
[2] *Compt. rend. Soc. de biol.*, Paris, 1895, p. 639.
[3] *Bull. Muséum d'hist. nat.*, Paris, 1896, t. II, p. 100.

preliminary measure, deprives this conclusion of the degree of certainty one would like to have in such a matter. On the other hand, the greater susceptibility of young hedgehogs prevents us from putting the immunity of the adult in the category of natural immunity properly so called.

Analogous considerations apply in the case of the mongoose [356]
(*Herpestes ichneumon*), carefully studied by Calmette[1], according to whose researches the Antilles mongoose is not very susceptible to snake venom; it readily withstands doses very large relatively to its size, but its immunity is not absolute. It owes much of its mastery in its fights with venomous snakes to its extraordinary agility. The blood of the mongoose, mixed with venom, exhibits an undoubted antitoxic power, though this is not sufficient to prevent the death of susceptible animals. We have no data to enable us to explain the origin of this antitoxic property, but it is probable that here again we have an example of relative immunity, acquired during life. Calmette points out, however, that his ichneumons came from Guadeloupe, where no venomous snakes are found. We may, of course, suppose that the feebly antitoxic power of the blood of these mammals might be due to other snakes or to species of animals whose blood possesses a certain venomous property[2].

We have far more exact data on the natural immunity of certain mammals against toxins of microbial origin. The example most thoroughly studied, one which has become, one might say, classic, is that of the rat against diphtheria toxin. Since the discovery of this toxin, the first well-studied bacterial poison, a discovery made by Roux in collaboration with Yersin, it has been recognised that mice and rats tolerate large quantities of diphtheria cultures or of their filtered products. A rat resists a dose of the diphtheria poison capable of killing several rabbits. To explain this great natural immunity it was suggested that the antitoxic property of the body fluids could be called in. It was supposed that the rat's blood was, by its very nature, endowed with the power of neutralising the

[1] "Le venin des serpents," Paris, 1896, p. 43.

[2] The temporary immunity of the marmot (amongst mammals) against tetanus toxin must be considered separately. According to Billinger and Dönitz the marmot is insusceptible to this poison during its winter sleep. But once it is awakened it readily contracts tetanus. H. Meyer, Halsey and Ransom have observed the same fact in hibernating bats that have been waked up. In these cases the immunity is dependent on the low temperature which approximates these examples to that of the natural immunity of the frog against the same toxin.

toxin of diphtheria. But, as in the tetanus of fowls, it was not long
[357] before facts rendered this hypothesis untenable. Kuprianow[1] studied this question under the direction of Loeffler and gave an account of the results of his experiments, which proved that the blood of the sewer rat, which is very refractory against diphtheria, contains no substance that will neutralise the morbific action of diphtheria toxin on susceptible animals, especially the guinea-pig.

It was necessary to seek some other explanation, and the idea that the immunity of the rat depends on the insusceptibility of its living cells to the diphtheria poison was seized upon. The experiments carried out by Roux and Borrel[2] demonstrated the incorrectness of this hypothesis. The immunity of rats to subcutaneous or intra-peritoneal injection of diphtheria toxin is very marked. But a very small dose (0·1 c.c.) of this poison, introduced directly into the cerebral substance of the rat, produces a complete paralysis, which lasts for several days, and ends in the death of the animal. Roux and Borrel conclude from this "that the brain of the rat is specially sensitive to the action of the diphtheria poison, and that as this animal does not die as the result of the injection of large quantities of toxin into the subcutaneous tissue, it is because the toxin does not reach the brain." These authors have pointed out analogous facts in connection with other examples of natural immunity. The rabbit, which withstands a hypodermic injection of 30 centigrammes of chlorhydrate of morphia, is killed by 1 milligramme only of this salt, introduced directly into the brain. Here, again, neither the cellular insusceptibility nor the antitoxic property of the blood (no "antialkaloidal" power could ever be demonstrated) can explain the immunity, which appears to be due rather to the factor which arrests the poison on its way to the nerve centres.

In spite of the insufficiency of our knowledge as regards natural immunity against soluble poisons we are quite justified in affirming that this category of phenomena comes mainly into the domain of the cells. The body fluids of animals which exhibit this immunity have been found to be antitoxic in a few species only (scorpion, snake, hedgehog, mongoose). And for the majority of these it is possible to invoke special causes, such as the internal secretion of snake and scorpion venoms by the glands which manufacture them, or the acquisition of an antitoxic power during life resulting from

[1] *Centralbl. f. Bakteriol. u. Parasitenk.*, Jena, 1894, Bd. XVI, S. 415.
[2] *Ann. de l'Inst. Pasteur*, Paris, 1898, t. XII, p. 225.

wounds or from the absorption of venomous food. The theory of [358]
the insusceptibility of the cells of animals naturally refractory to toxins must also be rejected ; it is incompatible with well-established facts. Nothing remains, then, but to assume that the formed elements are the principal factors in this natural immunity, and that they interpose to prevent the passage of the poisons towards the very susceptible nerve cells.

[359]

CHAPTER XII

ARTIFICIAL IMMUNITY AGAINST TOXINS

Adaptation to poisons.—Artificial immunity against bacterial and vegetable toxins and against snake venom.—Principal methods of immunisation.—Immunisation by toxins and toxoids.—Inoculation against diphtheria toxin.—Phenomena produced in the course of vaccination against toxins.—Rise of temperature.—Leucocytosis.—Development of antitoxic power.—Properties of antitoxins.—Mode of action of antitoxins.—Action of antitoxins *in vitro*.—Their action in the organism.—Influence of living elements on the combination of antitoxin with toxin.—Antitoxic action of non-specific serums, of normal serums and of broth.—Immunity against toxins is not in direct ratio to the amount of antitoxins in the body fluids.—Hypersensitiveness of an animal treated with toxin.—Diminution of the susceptibility of the organism immunised against toxins.

Hypotheses as to the nature and origin of antitoxins.—Hypothesis of the transformation of toxins into antitoxins.—Hypothesis of receptors detached from cells as the source of antitoxins.—Hypothesis of the nervous origin of tetanus antitoxin.—Fixation of tetanus toxin by the substance of the nerve centres.—The relations between saponin and cholesterin.—Anti-arsenic serum.—Part played by phagocytes in the struggle of the animal against poisons.—Probable part played by phagocytes in the production of antitoxins.

ALTHOUGH scientific men succeeded only a little more than ten years ago in vaccinating against poisons by artificial methods, savage races and ancient peoples at a very remote period undoubtedly possessed methods of counteracting the effects of certain venomous substances. The frequent observation of cases in which doses of poisons, insufficient to cause death, brought about a more or less durable resistant condition, must result in the elaboration of artificial means of preventing the intoxications.

Von Behring[1] points out that analogous facts must have been known to the physicians of ancient times; and it is in such knowledge that we must look for the source of the dogma put forward by Hippocrates, that the factor which produces a disease is also capable of curing it.

[1] "Allgemeine Therapie der Infectionskrankheiten," Berlin u. Wien, 1899, S. 982.

To Pliny we are indebted for the now well-known story, that Mithridates of Pontus possessed the means of protecting himself against various poisons by a process of adaptation, and, amongst [360] others, by the use of the blood of Pontine ducks to which he had given poisons by the mouth.

The adaptation of horses and of the highlanders of Styria to arsenic, as well as that of the many morphinomaniacs to morphia, is known to everybody. A man, habituated to morphia, is able to consume daily a dose several times the fatal one; indeed, cases have been known of people acquiring the power of consuming two, and even three, grammes of morphia per diem. Man may acquire an adaptation to toxic substances of the most diverse character, such as arsenic, alcohol, morphia, nicotine, etc.

Even when we had obtained much information concerning acquired immunity against micro-organisms we still knew nothing of the mechanism of such adaptation, or as to the possibility of acquiring a special immunity against bacterial poisons. Charrin and Gamaleia's discovery that animals vaccinated against a micro-organism are just as susceptible to its toxic products as normal animals, led Bouchard[1], in whose laboratory it was made, to say that the idea of the adaptation of cells to bacterial poisons must be dropped. He developed this thesis at the International Congress at Berlin in 1890, and formulated it as follows: "When we inject a healthy animal and a vaccinated one with the soluble products of the micro-organism which has been used for the vaccination, the dose required to kill each animal is exactly the same. Let us not speak, then, of the training of the leucocytes, and of the adaptation of the nerve cells to bacterial poisons: it is pure rhetoric." At this time we had only just commenced to acquire exact knowledge concerning the toxins of micro-organisms. For a considerable period they were sought for amongst the ptomains, very stable substances allied to the alkaloids; here, however, we were working in a wrong direction. It was not until the classic researches of Roux and Yersin[2] on diphtheria toxin, published in 1888 and 1889, that the true nature of bacterial poisons was revealed. It was found that we were not dealing with ptomains, but with soluble ferments, substances of indeterminate chemical composition, allied to the albuminoids, and, like them,

[1] "Essai d'une théorie de l'infection," Berlin, 1890; "Les microbes pathogènes," Paris, 1892, p. 33.

[2] *Ann. de l'Inst. Pasteur*, Paris, 1888, t. II, p. 629; 1899, t. III, p. 273.

[361] unstable. The methods adopted by Roux and Yersin in their study of diphtheria toxin enabled other investigators to discover the analogous toxins of several other bacteria. Knud Faber[1] and Brieger and Fränkel[2] soon succeeded in separating the toxin from the tetanus bacillus, a toxin capable of producing in animals tetanic contractions as typical as those obtained with cultures of the tetanus bacillus.

These investigations inaugurated a new era in microbiology and enabled us to attack the problem of acquired immunity against bacterial toxins scientifically. Within a few months of the declaration made by Bouchard at the Berlin Congress, there appeared, almost simultaneously, the earliest publications on the possibility of vaccinating laboratory animals against the toxins of diphtheria and tetanus by artificial methods. Immediately after the discovery of these poisons, the attempt was made to immunise various species of animals against them, but here very great difficulties were met with; the animals, after receiving increasing doses of toxin, became thin and ultimately died. It occurred to Fränkel[3] that the toxic action of the diphtheria poison might be weakened by subjecting it to a temperature of 60° C. Independently, von Behring and Kitasato[4] used chemical substances, especially iodine trichloride, to attenuate the action of the tetanus and diphtheria toxins. The animals which resisted these modified poisons were found to be capable of tolerating gradually increasing doses of unaltered and very active toxins. By the use of these methods it was found possible to obtain a definite and lasting immunity against these microbial products.

The discovery of the possibility of vaccinating against bacterial toxins was soon followed by the demonstration of the antitoxic power of the blood of animals that had acquired such artificial immunity against these poisons. Everyone knows of and appreciates von Behring and Kitasato's great discovery. It opened up a new and fruitful field of research from most diverse points of view. Ehrlich[5] was able to [362] apply it to the vaccination of animals against the vegetable poisons ricin, abrin and robin, and thus to establish rigorous methods of immunisation and to obtain very important results concerning immunity against toxins in general. He also succeeded in demonstrating that

[1] *Berl. klin. Wchnschr.*, 1890, S. 717.
[2] *Berl. klin. Wchnschr.*, 1890, No. 11.
[3] *Berl. klin. Wchnschr.*, 1890, No. 49.
[4] *Deutsche med. Wchnschr.*, Leipzig, 1890, SS. 1145, 1245.
[5] *Deutsche med. Wchnschr.*, Leipzig, 1891, SS. 976, 1218.

animals vaccinated against these vegetable poisons, which, by their nature, approximate to the microbial toxins, develop in their blood a most marked antitoxic property.

Some years later, the discovery of antitoxins was extended to snake venoms, poisons of animal origin which, like the vegetable poisons studied by Ehrlich, present a chemical composition analogous to that of the microbial toxins. Phisalix and Bertrand[1] and Calmette[2], working independently, discovered methods of vaccination against snake venom and were able to demonstrate the existence of an antitoxic power of the blood in immunised animals.

The works above briefly referred to gave us the fundamental basis of our present knowledge on acquired immunity against toxins.

It would be very interesting to be able to determine whether the lower animals can be vaccinated against the toxic substances to which they are susceptible. Unfortunately in the study of this problem we encounter very great difficulties. Making use of various methods I have often tried to solve it. The crayfish is susceptible to snake venom and to the ichthyotoxin of eel's serum, and I have tried at various times to vaccinate it against these poisons. The results, however, were so inconstant and even contradictory that I was unable to draw any definite conclusion from them.

It is, indeed, very difficult to vaccinate the lower vertebrata against poisons. Several attempts have been made in my laboratory to immunise frogs against tetanus toxin, but without success. Calmette and Deléarde[3] obtained the best results with abrin. They succeeded in vaccinating frogs—which are not very susceptible to this vegetable toxin, though they are far from presenting a real natural immunity—against doses which are absolutely fatal for the control animals. These observers, however, had to proceed very cautiously, and they allowed a very long interval between each injection of abrin. The blood of their vaccinated frogs not only did not prove to be antitoxic against [363] abrin, when injected into mice, but for long retained sufficient of this toxin to poison normal mice. This experiment certainly tells against the hypothesis that the acquired immunity of frogs is due to the development of a specific antitoxic power in their body fluids, but it does not settle the question definitely since it may be objected that

[1] *Compt. rend. Soc. de biol.*, Paris, 1894, p. 111.

[2] *Compt. rend. Soc. de biol.*, Paris, 1894, pp. 120, 204. [Cf. also Fraser, *Brit. Med. Journ.*, London, 1895, Vol. I, p. 1309 and II, p. 416; *Nature*, London, 1896, Vol. LIII, p. 571.]

[3] *Ann. de l'Inst. Pasteur*, Paris, 1896, t. x, p. 683.

the blood, whilst toxic for mice, might, still, be antitoxic for the frog. The antitoxin of this blood might merely be incapable of neutralising all the abrin present. Fresh investigations, then, are necessary.

Even in the higher vertebrata, it is often very difficult to obtain a real vaccination against the various toxins. In the small mammals, which exhibit a great susceptibility to these poisons, it is specially difficult to obtain an artificial immunity. As Vaillard and von Behring have demonstrated, it is possible to vaccinate such animals by means of gradually increasing doses of unmodified toxins, but this method demands much time, is often dangerous, and hence is not very practical. Poisons that act through the alimentary canal are the most serviceable for vaccination, as has been demonstrated by Ehrlich. This investigator had to abandon the vaccination of mice by means of subcutaneous injections of ricin on account of the sloughing set up at the point of inoculation. He then had recourse to vaccination by way of the mouth, which gave very good results, not only with ricin but also with abrin. This mode of vaccination, however, is applicable to a small number of poisons only.

We can also vaccinate mammals, even laboratory rodents, such as rabbits and guinea-pigs, by means of unmodified snake venom, but this method is a very delicate one and must be carefully watched. It is necessary to begin with very small doses of venom, continue them for some time, and increase the amount of venom injected very slowly. Calmette[1] modified this method by inserting, below the skin and leaving it there, a piece of chalk impregnated with small quantities of venom and surrounded by collodion through which the venom diffuses very slowly and continuously.

[364] Large mammals, sheep, oxen and horses, can be more easily vaccinated by means of unmodified toxins, but they also require to be treated with very special precaution. Salomonsen and Madsen[2] have given the history of their horse, immunised with diphtheria toxin. Into a mare weighing 665 kilos they were able to inject at the commencement only 1 c.c. of this toxin, and the dose had to be increased very carefully.

In the presence of all these difficulties in the use of unmodified toxins for vaccination, a different method is now generally adopted in the immunisation of animals, small or large, for the purpose of scientific research or for the preparation of toxins on a commercial

[1] "Le venin des serpents," Paris, 1896, p. 54.

[2] *Ann. de l'Inst. Pasteur*, Paris, 1897, t. XI, p. 316.

scale. Vaccination is commenced with toxins modified by heat or by chemical substances. The diphtheria and tetanus toxins, those most employed in the serotherapeutic industry, are subjected to various degrees of heat. Fränkel[1] was the first to make use of this method for vaccination against diphtheria, and Vaillard[2] for vaccination against tetanus. It consists in introducing large doses of filtered cultures, heated to progressively lower degrees of temperature, 60°, 55°, 50° C., and then giving gradually increasing quantities of filtered cultures whose toxicity is unaltered. This method is very convenient for small animals, but for large mammals it is greatly simplified by injecting for a certain period toxins heated to 60° C., and, later, replacing these by unmodified toxin.

Phisalix and Bertrand[3] applied an analogous method to the vaccination of the guinea-pig against the venom of the viper. This poison, which resists much higher temperatures than do the tetanus and diphtheria toxins, received a preliminary heating to 80° C. in order that it might be inoculated without danger into small animals. Under these conditions it confers a certain immunity, but even when heated to 80° C. it, in many cases, still remains sufficiently active to produce fatal results. For this reason, in the vaccination of animals for the preparation of antivenomous serum on a large scale, Calmette had recourse to another method, that of attenuating the venom by [365] means of chemical substances.

Von Behring and Kitasato[4] were the first to make use of iodine trichloride in the vaccination of animals against the toxins of tetanus and diphtheria. In their early experiments this substance was injected before the toxins were introduced. Later, the mixture was made *in vitro* and then injected into the animals. Roux devised another method which had the advantage of being simple, certain, and easily employed, for which reason it was soon introduced into commercial and scientific practice. It consists in the injection of mixtures of the tetanus or diphtheria toxins with Lugol's iodo-ioduretted solution. The iodine, in small doses, instantly neutralises or modifies these poisons and is itself borne well, even by small animals. By employing progressively increasing doses of these mixtures, in which the amount

[1] *Berl. klin. Wchnschr.*, 1890, No. 49.

[2] *Ann. de l'Inst. Pasteur*, Paris, 1892, t. VI, p. 225.

[3] *Compt. rend. Acad. d. sc.*, Paris, 1894, t. CVIII, p. 288; *Compt. rend. Soc. de biol.*, Paris, 1894, p. 111.

[4] *Deutsche med. Wchnschr.*, Leipzig, 1890, SS. 1145, 1245.

of iodised solution becomes smaller and smaller compared with that of the toxin, we are able, without difficulty, to vaccinate the most susceptible animals and enable them to withstand considerable doses of the pure toxin. By this method it is possible to immunise guinea-pigs against the most active tetanus toxin. The method serves equally well for the preparation of horses for injections of unmodified toxins. For a longer or shorter time (according to the susceptibility of the horse) toxins which are mixed with Lugol's iodised water are injected. Having made sure of the resistance of the horse, larger and larger quantities of pure, unmodified toxin may be introduced with impunity.

For the immunisation of mammals of all sizes (guinea-pigs, rabbits, dogs, horses) against snake venom, Calmette, in his work at Lille, also makes use of venom modified by chemical substances, but his method differs from those we have just described. During several weeks he injects increasing quantities of venom, mixed with decreasing quantities of a solution of 1 : 60 of hypochlorite of lime. After this treatment the animals become capable of tolerating fatal doses of unmodified venom and can be injected with larger and larger doses.

In recent years a method of vaccinating horses against certain microbial toxins, and especially against the diphtheria toxin, by means of mixtures of toxin and antitoxic serum, or with these two products successively, has been introduced. Babes[1] was the first to extol
[366] this method as the best for obtaining a high and durable immunisation. Afterwards, several other observers, amongst whom I may cite Pawlowsky and Maksutow[2], Palmirsky, and especially Nikanoroff[3], took up this question, and communicated very encouraging results. Von Behring[4] also found it very useful in certain cases. Thus, for the vaccination of guinea-pigs against tetanus toxin, he recommends the injection of a mixture containing antitoxin and an unneutralised excess of toxin. Under these conditions he easily succeeds in immunising these small animals in cases where all other methods fail. As a general method of vaccination against toxins, however, this method has not fulfilled its promise, and Roux, who tried it several times, was not at all satisfied with it.

[1] *Bull. Acad. de méd.*, Paris, 1895, t. XXXIV, p. 216.

[2] *Ztschr. f. Hyg.*, Leipzig, 1896, Bd. XXI, S. 485.

[3] "On the preparation of a potent antidiphtheria serum," St-Pétersbourg, 1897 (in Russian) [cf. *Berl. klin. Wchnschr.*, 1897, S. 720].

[4] "Allgemeine Therapie der Infectionskrankheiten," Berlin u. Wien, 1899, S. 1093.

This method of immunisation by mixtures of toxin and antitoxin is often spoken of as the method of vaccination by *toxones*. This name, "toxone," was first applied by Ehrlich[1] to a product developed by the diphtheria bacillus in culture media, a product less and differently toxic than is the true diphtheria toxin, yet capable of neutralising antitoxin. The idea of toxones presented itself to Ehrlich in connection with a fundamental fact noted by him, namely, that when to a non-toxic mixture of diphtheria toxin and antitoxin there is added one and even several lethal doses of the former, the animal is not affected. To make it succumb to intoxication it is sometimes necessary to add more than 20 lethal doses of toxin. To explain this paradoxical result, Ehrlich formulated the hypothesis that, in the soluble products of the diphtheria bacillus there exist two poisons: (1) the true toxin which exhibits a very strong affinity for antitoxin, and (2) the toxone which possesses less avidity for this antibody. When to an inactive mixture of the products of diphtheria bacilli and of antitoxin, there is added a fresh quantity of these same products, the added toxin, owing to its greater affinity, replaces the toxone of the previous combination. In the mixture to which is added one or several lethal doses of diphtheria poison, the toxone [367] only is found free, all the toxin being combined with the antitoxin, and, as the toxone is only feebly toxic, the animal resists without suffering any serious illness.

Madsen[2] adopted the theory of the diphtheria toxone, and affirmed that this substance poisons but slowly, produces neither early nervous symptoms nor loss of hair, but excites slight oedema at the point of inoculation and late paralyses. Susceptible animals may die from toxones, but very much later than as the result of poisoning by the toxins.

Ehrlich's pupils have extended the theory of toxones to other bacterial poisons. Thus Madsen[3] has described a similar toxone in tetanus poison—the tetanolysin of Ehrlich—which dissolves the red blood corpuscles, and Neisser and Wechsberg[4] refer to a toxone in the poison produced by the staphylococcus.

Ehrlich also describes *toxoids* as occurring in diphtheria poison. The toxone, he maintains, is a product of the diphtheria bacillus

[1] *Deutsche med. Wchnschr.*, Leipzig, 1898, S. 597.
[2] *Ztschr. f. Hyg.*, Leipzig, 1897, Bd. XXIV, S. 425.
[3] *Ann. de l'Inst. Pasteur*, Paris, 1899, t. XIII, pp. 568, 801.
[4] *Ztschr. f. Hyg.*, Leipzig, 1901, Bd. XXXVI, S. 325.

itself, but the toxoids (protoxoids and syntoxoids) represent the toxin modified without further aid from the bacillus. The toxoids, though not toxic, retain all their avidity for antitoxin. According to Ehrlich's conception, the molecule of toxin, under the influence of various factors, readily loses its toxic or *toxophore* group, capable of poisoning the animal, whilst still retaining its *haptophore* group, the group that combines with the antitoxin. The toxoids then would represent this haptophore group of the diphtheria toxin. Without being injurious to animals, the toxoids are capable of neutralising the antitoxin and of setting up in the animal the formation of this antibody. In the experiments carried out by the method of Babes and of the Russian authors we have just mentioned, there would be, according to the view held by Ehrlich and his school, an immunisation by the toxoids.

The toxones, however, are also capable of vaccinating against the toxin and the toxone and of giving rise to the production of a diphtheria antitoxin, active against these two poisons. This is what
[368] is affirmed by Madsen[1] and by Dreyer[2], according to a communication made by the latter to the International Congress of Medicine held at Paris.

By means of the various methods briefly described above, is obtained a real acquired immunity against the various bacterial and vegetable poisons and the venoms. On the other hand, with the methods of vaccination mentioned in the eighth chapter, which confer a substantial immunity against micro-organisms, we cannot demonstrate, in the vaccinated animals, a resistance against the corresponding toxins greater than in the unvaccinated control animals. The animals, so thoroughly vaccinated against certain micro-organisms that they withstood enormous doses of culture, did not become capable of resisting the minimal lethal dose of the poison. We are led to conclude, therefore, that immunity can only be obtained against certain of the toxins. For this reason we must regard the attempt made by von Behring to obtain a real immunisation against the toxin of cholera as an important forward step. Before von Behring's attempt, various species of animals had been frequently and very substantially vaccinated against the cholera

[1] *Compt. rend. du Congrès internat. de méd. de Paris* (Section de bactériologie et parasitologie), 1901, p. 40.

[2] *Compt. rend. du Congrès internat. de méd. de Paris* (Section de bactériologie et parasitologie), 1901, p. 45 ; *Ztschr. f. Hyg.*, Leipzig, 1901, Bd. XXXVII, S. 250.

vibrio, but these animals, even when most thoroughly vaccinated, were completely non-resistant to the cholera toxin. Von Behring suggested to his pupil Ransom[1] the idea of immunising guinea-pigs, not with microbial cultures living or dead, as had usually been done previously, but exclusively with the fluids of the cultures, deprived of the vibrios by filtration. In order, however, to attain the desired object, it was necessary to prepare fluids sufficiently active to poison the unvaccinated control guinea-pigs with certainty. The results of these investigations confirmed his anticipation, and Ransom soon found himself in possession of guinea-pigs well vaccinated against the cholera poison. He was mistaken, however, in supposing that, in all cases of immunity acquired against Koch's vibrio, we have to do, in the main, with a purely antitoxic immunity. An investigation carried out in the Pasteur Institute[2], whilst confirming the facts discovered by Ransom, lead to different results as regards their interpretation. It was demonstrated that the immunity against the vibrio is in no [369] way founded on a resistance against its toxin and that we have to do with two very different acquired immunities. The vaccination obtained with the bodies of the micro-organisms induced a refractory condition against infection by the living vibrio, but not the slightest resistance against the toxin. The immunity, on the other hand, which is conferred by the injection of soluble products, deprived of the micro-organisms, is effective not only against the toxin of cholera, but also against infection by the vibrio. When an animal is vaccinated with cultures, or even with the bodies only of the vibrios, cholera toxin is introduced, but the toxin, under these conditions, is incapable of setting up antitoxic immunity. It would appear that the presence of the vibrios may constitute some obstacle to the production of this immunity.

Soon afterwards, Wassermann[3] pointed out that the same rule applies in the case of the *Bacillus pyocyaneus*. With whole cultures of this bacillus he obtained in guinea-pigs an immunity exclusively against infection, whilst with cultures in a fluid medium, deprived of the bacilli, he was able to vaccinate his animals both against the pyocyanic toxin and against the infective peritonitis produced by the living micro-organism. The same double immunity could also be obtained

[1] *Deutsche med. Wchnschr.*, Leipzig, 1895, S. 457.
[2] *Ann. de l'Inst. Pasteur*, Paris, 1896, t. x, p. 257.
[3] *Ztschr. f. Hyg.*, Leipzig, 1896, Bd. xxii, S. 312.

in laboratory animals against the typhoid bacillus and several other bacteria.

When animals were subjected to different methods of vaccination against toxins, the manifestation of certain phenomena more or less constant was observed; amongst these must be pointed out especially the rise of temperature, a local reaction and certain modifications in the body fluids.

Fever is a very general symptom in the course of the vaccination of mammals. A rise of temperature is almost always observed as a result of the injection of toxins. It is very variable, both as regards duration and intensity, and cannot serve as an indicator of the result of the vaccination. In this respect, such great differences have been observed that the attempt to establish any general laws has had to be abandoned.

Local reaction is also a phenomenon which is very frequently observed during vaccination; to this von Behring[1] paid great attention. He and his collaborators found that normal horses when
[370] injected subcutaneously with small or large doses of tetanus toxin did not present any exudation at the seat of inoculation. The horses which died as the result of a tetanus intoxication and those which got better behaved from this point of view in much the same fashion. In horses, however, which are being vaccinated and which are periodically subjected to gradually increasing doses of toxin, tumefaction at the seat of injection is never absent. Von Behring attributes this difference to the primordial insusceptibility of the living elements which govern exudation in the subcutaneous tissue to tetanus poison. It is only during the process of vaccination that these cells become susceptible and capable of manifesting a visible reaction. I consider that this difference is due more probably to a change in the chemiotaxis of the various elements which contribute to the inflammatory exudation reaction, from a negative to positive type. The cells do not react at the commencement, not because they are not susceptible to the toxin, but rather because their susceptibility is too great. During the course of vaccination they become sufficiently adapted to the poison to be able to manifest their normal inflammatory reaction. This explanation certainly harmonises with the fact that during the period of vaccinations in general and of vaccination against toxins in particular, the blood usually presents a more or less distinct hyperleucocytosis. Now, as is well known, this

[1] "Allgemeine Therapie der Infectionskrankheiten," Berlin u. Wien, 1899, S. 1052.

phenomenon of hyperleucocytosis is one of the most striking manifestations of a positive chemiotaxis in white corpuscles. It is true that, as to this reaction during the course of vaccination, the views of observers are not unanimous. Besredka[1], as the outcome of his work on this subject, expresses himself very distinctly. "During the course of an immunisation against diphtheria toxin," he writes, "one always observes a marked reaction in the goat, either at the beginning or at an advanced stage of the period of injections and especially in the first few hours after injection" (p. 322). Nicolas and Courmont[2] in their first memoir maintain that hyperleucocytosis "is not necessary for immunisation." Nevertheless, in the description of their experiments, which were performed on horses vaccinated against diphtheria, it is clear that the number of white corpuscles is often markedly increased. Further, in several cases they describe the formation of tumours at the point of inoculation, [371] some of which end in suppuration. Under these conditions, it is not possible to deny a vaccinal reaction on the part of the leucocytes. Later, Nicolas, Courmont and Prat[3] published a second memoir on the same subject, in which they seek to confirm their view of the uselessness of hyperleucocytosis in vaccination against the poison of diphtheria. They give details of experiments on several species of animals and insist specially on the conditions in which they have not observed hyperleucocytosis. "The doses from the first have always been extremely weak and with the addition of Lugol's solution to attenuate them; only very gradually have we reached stronger doses, as *that is one of the indispensable conditions for the avoidance of leucocytic variations*, whilst obtaining a good and rapid immunisation" (p. 974). These special precautions to avoid hyperleucocytosis demonstrate clearly that this phenomenon is usually produced during the course of vaccination. It is quite natural that we should, by proceeding very slowly and with small doses of toxin, succeed in diminishing or even suppressing the afflux of leucocytes; but this fact cannot in any way minimise the importance of the leucocytic reaction in vaccination. In these particular cases, this reaction may take place without the number of leucocytes in the blood being noticeably increased. In reading the details of the experiments made by the Lyons observers, it will be seen that,

[1] *Ann. de l'Inst. Pasteur*, Paris, 1898, t. XII, p. 318.

[2] *Arch. de méd. expér. et d'anat. path.*, Paris, 1897, t. IX, p. 770.

[3] *Journ. de physiol. et de path. gén.*, Paris, 1900, t. II, p. 973.

in spite of all their precautions, they were unable to prevent the production of hyperleucocytosis. In all their cases, where they took the precaution to count the leucocytes several times a day, there was an undoubted increase of these cells. We may here recall Salomonsen and Madsen's account of the immunisation of a horse against diphtheria toxin, in which they point out the frequency of tumefactions and even of abscesses. In most cases the pus was sterile, which renders it probable that the white corpuscles had accumulated at the seat of inoculation as the result of some influence exerted by the diphtheria toxin.

By far the most important and remarkable change met with in animals vaccinated against toxins and venoms, consists in the appearance of antitoxic power in their blood and fluids in general. This
[372] fact was, as already mentioned, first demonstrated by von Behring and Kitasato[1] in the blood of rabbits immunised against tetanus. The blood itself, or the blood serum, mixed with a quantity of tetanus toxin more than sufficient to cause fatal poisoning, sets up no disease when injected into animals. In their earliest researches, von Behring and Kitasato kept the mixtures in contact *in vitro* for 24 hours, before injecting them into test animals. Later, they found that this prolonged contact outside the body was unnecessary and that they could obtain successful results by injecting the serum of vaccinated animals and the toxin simultaneously, even at different points of the body. This discovery was immediately afterwards applied by its authors to diphtheria and, in the case of both intoxications, confirmed by numerous observers.

For some time we were satisfied with vaccinating small laboratory animals and establishing the antitoxic power of their blood serum; later, the vaccination of large animals, especially horses, was commenced with the object of obtaining large quantities of antitetanus and antidiphtheria serum for medical use. During the course of these experiments the principal characters of the antitoxic fluids were established. It was deemed desirable to isolate the antitoxic substance from the blood serum in order to get rid of every unnecessary and inactive admixture, so that the antitoxin might be used in as pure a form as possible. This idea of isolating the antitoxic substance had, however, soon to be abandoned as impossible of realisation. Antitoxin is a non-crystallisable substance, of unknown chemical composition, which adheres firmly to the albuminoid

[1] *Deutsche med. Wchnschr.*, Leipzig, 1890, S. 1113.

substances of the serum. It is usually regarded as belonging to the same albuminoid group of substances, though it is not possible to prove this satisfactorily. Von Behring[1], however, who studied this question in collaboration with Knorr, denies the albuminoid nature of tetanus antitoxin. After demonstrating that this antitoxin, when the antitetanus serum is submitted to dialysis, passes through the dialysing membrane, these observers found that they could not obtain the characteristic reactions of albuminoids in the dialysed fluid. It must be admitted, however, that this negative result is not sufficient to justify a denial of the albuminoid nature of [373] antitoxin. When Nencki and Mme Sieber[2] sought to produce the reactions of albuminoid substances with the digestive juice of *Nepenthes* (the well-known insectivorous plant) they got no result; but after the concentration of the juice *in vacuo*, it at once gave the characteristic reaction with nitric acid, and also with acetic acid, potassium ferrocyanide and Millon's reagent.

The antitoxins may be precipitated along with the globulins and are distinguished, in general, by a fairly great resistance against physical and chemical influences. In this respect they are allied to the agglutinins, the fixatives and the precipitins, considered elsewhere, and are sharply distinguished from the cytases. The antitoxins resist temperatures which destroy the cytases and remain unaltered to beyond 60°—65° C. They are more stable than the delicate toxins of tetanus and diphtheria, but they are more easily altered than the toxins of cholera, of *Bacillus pyocyaneus* and the venoms. When stored in a dry state in the residue of evaporated serums and protected from light and air, the antitoxins will keep for a very long time without showing any notable attenuation. This property is very important in practice.

The antitoxins, in this respect also resembling the fixatives and the agglutinins, are humoral substances in the strictest sense of the term. They are found not only in prepared serums but abound also in the plasma of the circulating blood, and in the plasmas of the lymph and of exudations. Vaillard and Roux[3] have shown that the clear acellular serous fluid of the oedema produced by the slowing of the circulation in rabbits vaccinated against tetanus toxin, is as antitoxic as the blood itself. Even the aqueous humour of a strongly

[1] "Die praktischen Ziele der Blutserumtherapie," Leipzig, 1892, S. 52.

[2] *Ztschr. f. physiol. Chem.*, Strassburg, 1901, Bd. XXXII, S. 318.

[3] *Ann. de l'Inst. Pasteur*, Paris, 1893, t. VII, p. 81.

immunised animal is antitoxic, though to a less degree. On the other hand, the saliva and urine exhibit very little antitoxic power, even when they are derived from animals hyperimmunised against tetanus toxin. Milk, as first demonstrated by Ehrlich[1], is fairly rich in antitoxin, although much less so than the blood. According to the estimation of Ehrlich and Wassermann[2], in the same immunised
[374] animal, milk contains one-fifteenth to one-thirtieth of the amount of diphtheria or tetanus antitoxin contained in the blood. Pus is always less antitoxic than blood or blood serum. According to Roux and Vaillard (*l. c.*, p. 82), the pus of their rabbits vaccinated against tetanus toxin was only one-sixth or one-eighth as antitoxic as the serum of the blood. In Salomonsen and Madsen's[3] antidiphtheritic horse the cellular sediment of the pus was about one-half as antitoxic as the blood.

For the development of the antitoxic property in the fluids of the body, it is not essential that animals should belong to species susceptible to the corresponding toxin. Animals naturally most refractory against the poisons of diphtheria and tetanus are also capable of producing antitoxins. Vaillard[4] demonstrated this fact in the fowl. This bird, which is naturally refractory against tetanus, usually acquires a very marked antitetanic power in its blood after one or more injections of tetanus toxin. He observed, however, that, in fowls thus treated, at a stage when the fluids of the body are antitoxic, the albumen of the egg is not so. The antitoxin, therefore, does not pass into this nutritive secretion, as it does into the milk of mammals. On the other hand, as has been demonstrated by F. Klemperer[5], the vitellus of the eggs of fowls treated with tetanus toxin in time acquires an antitoxic property of the most marked character.

The antitoxins, found especially in the fluids of the body but only scantily in the cells, exert some action on the toxins. What is the nature of this action? This question, much studied and discussed, is one of very great importance in connection with the general problem of acquired immunity against toxins. In his first memoir, written in collaboration with Kitasato, von Behring (*Deutsche*

[1] *Ztschr. f. Hyg.*, Leipzig, 1892, Bd. XII, S. 183.

[2] *Ztschr. f. Hyg.*, Leipzig, 1894, Bd. XVIII, S. 248.

[3] *Ann. de l'Inst. Pasteur*, Paris, 1897, t. XI, p. 324.

[4] *Compt. rend. Soc. de biol.*, Paris, 1891, p. 462; *Ann. de l'Inst. Pasteur*, Paris, 1892, t. VI, p. 229.

[5] *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1893, Bd. XXXI, S. 371.

med. Wchnschr., Leipzig, 1890, S. 1113) formulates his first thesis as follows: "the blood of a rabbit immunised against tetanus possesses the property of destroying tetanus toxin." This idea of destruction, which would remove all toxic power from the poison, would naturally present itself to the mind and was at once accepted by a great many observers, but the numerous facts now accumulated on the subject [375] will not allow us to accept a real destruction of toxins by antitoxins. Tizzoni[1] was one of the first to point out certain contradictions between the theory of destruction and the phenomena produced in animals injected with tetanus toxin and antitoxin. Buchner[2] also brought forward new facts which led him to conclude that antitoxin, instead of acting directly on the toxin, exerts its influence exclusively on the living elements, thus protecting the animal against intoxication. Amongst the arguments advanced by the Munich observer, the principal one is drawn from the different action of mixtures of tetanus toxin and antitetanus serum on various species of animals. It has been clearly shown that the guinea-pig is more susceptible to tetanus than is the mouse. In poisoning with tetanus toxin it requires an absolutely larger quantity of toxin to kill the guinea-pig than to kill the mouse. But if we take into account the weight of these animals, the conditions change completely. Thus, to cause a fatal tetanus in a guinea-pig, which weighs twenty times more than a mouse, we need only inject into the former a dose at most ten times greater than that necessary to produce fatal intoxication in the mouse. Buchner prepared a mixture of tetanus toxin and antitetanus serum which, in the mouse, produces no tetanic phenomenon or only sets up feeble and transient symptoms. According to the theory of direct action, we must assume that in this mixture the toxin is completely or almost completely neutralised by the antitoxin of the serum. But when Buchner injected the same quantity of mixture into guinea-pigs, without increasing it in proportion to the greater weight of these animals, he produced a tetanus of the most marked character. There has, consequently, remained in the mixture a sufficient amount of free toxin, whose tetanigenic action is manifested in the guinea-pig, an animal, as we have seen, more susceptible than the mouse. Buchner's experiment has been verified by several observers. Roux and Vaillard[3] carried out others which afford

[1] *Berl. klin. Wchnschr.*, 1893, S. 1266.
[2] *München. med. Wchnschr.*, 1893, S. 480.
[3] *Ann. de l'Inst. Pasteur*, Paris, 1894, t. VIII, p. 725.

similar evidence. The same mixture of tetanus toxin and specific serum which is borne without the least difficulty by normal guinea-pigs, causes typical tetanus in other guinea-pigs of the same weight, and apparently in the best of health, but which have been im-
[376] munised some time before against the Massowah vibrio. In another series of experiments, Roux and Vaillard injected into guinea-pigs a very large amount of antitetanus serum "capable of immunising them thousands of times," and, shortly afterwards, a lethal dose of tetanus toxin. The normal guinea-pigs were thoroughly resistant to this test, whilst several guinea-pigs into which were also injected the products of other micro-organisms, acquired tetanus. Analogous results were obtained with mixtures of diphtheria toxin and antidiphtheria serum. Roux concludes from these facts "that the antitoxins act on the cells." Against the theory of the destruction of toxins by antitoxins, he invokes the influence of heat on mixtures of these two substances. Calmette[1], under Roux's inspiration and in his laboratory, carried out various experiments on anti-venomous serum. A mixture of this with snake venom, in such proportion that the poison became inactive, regained its toxicity after being heated for five minutes at 68° C. A normal animal, injected with this mixture, succumbed as if it had received pure venom. On being heated at 68° C. the serum lost all its antitoxic power over the venom, and the latter, which only becomes modified at a much higher temperature, remained intact. Later, a similar result was obtained by Wassermann[2] in his experiments with pyocyanic toxin. This poison is resistant at even higher temperatures than is snake venom, whilst the antitoxin of the serum is destroyed under the same conditions as are the other antitoxins. Taking advantage of these peculiarities, Wassermann boiled the mixture of pyocyanic toxin and antitoxin serum, being careful to dilute it with two volumes of distilled water before doing so. This mixture which, before it was heated, was quite innocuous for guinea-pigs, again became a fatal poison after the destruction of the antitoxin.

These experiments prove clearly that, in the action of the antitoxin on the toxin, there can no longer be any question of an actual destruction of the latter, a view which has been accepted by both von Behring and Ehrlich. But, as pointed out by Roux at the

[1] "Le venin des serpents," Paris, 1896, p. 58.
[2] *Ztschr. f. Hyg.*, Leipzig, 1896, Bd. XXII, S. 263.

International Congress at Budapest in 1894, the manifestation of the toxic action of the venom after it has been heated along with antitoxin, may be reconciled with the view that the combination between the two substances, if such take place, must be very unstable. This same remark may be applied to Wassermann's experiment. [377] Therefore the great majority of observers, if not all, admit that the antitoxin combines with the toxin to form an innocuous and unstable substance which can be decomposed by heat and by other agents. The researches on the action of antitoxins *in vitro* have had a powerful influence in determining this view.

We have already in Denys and van de Velde's[1] experiments an indication of the direct action of certain antitoxins. These observers showed that the serum of animals vaccinated against a Staphylococcus is capable of neutralising *in vitro* a particular toxin to which van de Velde gave the name of *leucocidin*. When it was added to a drop of the exudation from a rabbit, this leucocidin in a very short time destroyed the white corpuscles, by dissolving the cell content but leaving the nucleus untouched. When Denys and van de Velde prepared mixtures of leucocytes, leucocidin and antileucocidic serum *in vitro*, the white corpuscles retained their normal condition for a very long time. The leucocidin was, therefore, rendered inactive by the direct influence of the corresponding antitoxin. These facts have been confirmed by Bail[2] and other observers and even extended to certain other microbial toxins. Thus, the *Bacillus pyocyaneus* produces a leucocidin which kills the white corpuscles and dissolves their contents[3]. With the object of facilitating experiments with these leucocytic poisons and the corresponding antitoxic serums, Neisser and Wechsberg[4], of the Institute of Experimental Therapeutics at Frankfort, invented a method which allows us to observe the phenomena of the destruction of the leucocytes and of the antitoxic power in test tubes, without having recourse to a microscopical examination. They applied the fact, discovered by Ehrlich, that living formed elements reduce methylene blue and, depriving it of its oxygen, decolorise it. Leucocytes from aseptic exudations are introduced into tubes and a weak solution (2%) of methylene blue is poured on them. To prevent the re-oxidation of this colouring-

[1] *La Cellule*, Lierre et Louvain, 1896, t. XI, p. 359; *Ann. de l'Inst. Pasteur*, Paris, 1896, t. X, p. 580.

[2] *Arch. f. Hyg.*, München u. Leipzig, 1897, Bd. XXX, S. 348.

[3] Gheorghiewsky, *Ann. de l'Inst. Pasteur*, Paris, 1899, t. XIII, p. 298.

[4] *Ztschr. f. Hyg.*, Leipzig, 1901, Bd. XXXVI, S. 330.

matter by the oxygen of the air, the surface of the fluid is covered
[378] with a layer of liquid paraffin. If the leucocytes are living, the lower blue layer becomes decolorised in a short time (in about two hours); when the corpuscles are dead, decoloration does not take place. By adding to the mixture of leucocytes and colouring matter some leucocidin, alone or along with antileucocidic serum, it is possible not only to observe with the naked eye the phenomena which take place in these cases, but also to estimate to some extent the proportions of poison and counterpoison.

All these researches make it clear that the antitoxin acts directly on the leucocidin. Similar facts have been noted as regards certain other organic poisons and their antitoxins. Shortly after the discovery of antileucocidin by Denys and van de Velde, Kanthack made a communication to the Physiological Society in 1896[1], exhibiting tubes in which the coagulating action of Cobra venom on the blood had been prevented by the addition of antivenomous serum. Of all the experiments, however, made to prove the direct action of antitoxin on toxin, Ehrlich's[2] have played the most important part in the study of this question. Ehrlich directed his attention to ricin which, as Kobert demonstrated, has the property of agglutinating the red corpuscles of defibrinated blood. This phenomenon can be easily observed *in vitro*. In tubes containing red blood corpuscles, the addition of ricin causes these corpuscles to agglutinate into clumps and to fall to the bottom of the tube, leaving a clear supernatant fluid. After adding progressively increasing quantities of antiricic serum to the tubes containing fluid blood and ricin, Ehrlich was able to demonstrate that small quantities of antiricin merely retarded the precipitation of the red corpuscles, whilst larger doses completely prevented it. Having studied the proportions of ricin and its antidote, necessary to retard and prevent the fatal poisoning of animals, Ehrlich was struck by the parallelism which is exhibited between the action of the antitoxin in the living animal and that in the test tubes.

The study of anticytotoxins, discussed in the fifth chapter, has furnished another opportunity of observing the action of antitoxins
[379] *in vitro*. Camus and Gley and H. Kossel were the first to observe the action *in vitro* of antitoxic serum against the ichthyotoxin of eel's serum. Since this observation, this phenomenon has been repeatedly studied in the antihaemolysins and antispermotoxins.

[1] [At a meeting held at St Bartholomew's Hospital, London, cited by Stephens and Myers in *Journ. Path. and Bacteriol.*, Edin. and London, 1898, vol. v, p. 280.]

[2] *Fortschr. d. Med.*, Berlin, 1897, Jahrg. xv, S. 41.

The antidiastatic serums also act *in vitro* and, as their effect can be demonstrated on soluble ferments placed in contact with unorganised bodies, such as gelatine and casein, the purely chemical character of the reaction is all the more strikingly shown. We are indebted to von Dungern, Briot and Morgenroth for accurate observations on this subject.

Martin and Cherry[1] made use of a different method to demonstrate the direct action of antitoxins on toxins which exhibit their toxic power on the animal organism. They chose snake venom mixed with antivenomous serum. The mixtures were filtered under great pressure [50 atmospheres] through a film of gelatine, under the idea that, if the venom and antitoxin were not chemically combined, the former alone, owing to its much smaller molecules as compared with those of the antivenom, would pass into the filtered fluid. This fluid should, under these conditions, possess a toxic power for animals, when the mixture, used for filtration, was deprived of the larger molecules. Martin and Cherry left the venom and the antitoxic serum in contact for periods of varying length, before filtering the mixtures. As the result of a series of such experiments carried out according to this scheme, they found that the product of the filtration made after some minutes' contact between the two substances, was distinctly toxic; whilst the filtrate obtained after a contact of half-an-hour was absolutely innocuous. From their observations these authors conclude that the antitoxin enters into chemical combination with the venom, but that the combination does not take place instantaneously, a certain amount of time being necessary for its accomplishment.

In addition to the time factor others have an influence on the combination between toxins and antitoxins, as is seen from Ehrlich's[2] and Knorr's[3] investigations. Both observers have shown that antitoxin neutralises the toxin more slowly in dilute solutions than in more concentrated form. For this reason, when animals are injected [380] with very weak solutions, the toxin may manifest its action before it can be neutralised by the antitoxin; this may lead to erroneous conclusions. On the other hand, according to data furnished by these authors, temperature also exerts an influence on the combi-

[1] *Proc. Roy. Soc. London*, 1898, Vol. LXIII, p. 423.

[2] *Klin. Jahrbuch.*, Berlin, 1897, Bd. VI, S. 13 [of reprint].

[3] *Fortschr. d. Med.*, Berlin, 1897, Jahrg. XV, S. 657; *München. med. Wchnschr.*, 1898, S. 321.

nation. Lowering the temperature retards, whilst raising it accelerates the neutralisation of the toxins by the antitoxins. Insisting on the purely chemical character of the combination between these two substances, Ehrlich and Knorr adduce the fact that this combination, in cases where we have a complete neutralisation of the toxin, follows, most rigorously, the law of multiple doses, that is to say, in order to render innocuous a hundred doses of toxin we have only to take a hundred times the quantity of antitoxin.

The series of facts summarised above demonstrate distinctly that antitoxins act directly on toxins. But how can this result be reconciled with the observations given above according to which must be admitted the no less real influence of the organism of the living animal on intoxication by mixtures of antitoxin with toxin? Knorr[1] sought at first to minimise the importance of the facts brought forward by Buchner and Roux. He failed to corroborate Buchner's results and found that the injection of mixtures, made with very large doses of tetanus toxin (20,000 times the minimal lethal dose) and corresponding quantities of antitetanus serum, brought about the same effect in guinea-pigs and mice. By modifying the quantity of antitoxin, he rendered the mixture equally innocuous or equally toxic for these two species. But the data given by Knorr are quite sufficient to prevent us from accepting his conclusion. In his experiments, as in those of Buchner, the guinea-pigs manifested a greater susceptibility and died from mixtures which, in mice, caused merely a tetanus of medium intensity.

Some have sought to explain Buchner's experiment by assuming that the mixtures, lethal for the guinea-pig and innocuous for the mouse, owed their toxic action to the presence of the *tetanus toxone* and not of the true tetanus poison, the *tetanospasmin*. This hypothesis of toxones, as stated above, was put forward by Ehrlich as the
[381] outcome of his ingenious researches on the constitution of the diphtheria poison. As, however, the toxones must act differently from the toxins, we can only attribute to their action the results in those cases where the guinea-pigs die without presenting typical symptoms of true tetanus, that is to say without spasms. Now, in Buchner's experiments, a much larger proportion of these animals, injected with the same mixtures as the mice, succumbed and exhibited the characteristic tetanic convulsions. Even in those cases, however,

[1] "Experimentelle Untersuchungen über die Grenzen der Heilungsmöglichkeit des Tetanus," Marburg, 1895, SS. 14, 21.

where the death of the guinea-pigs might be attributable to an intoxication by the toxone, the general result could not be altered. The toxones are, according to Ehrlich, manufactured by the micro-organisms in the culture media and form an integral part of the natural microbial poisons. Again, they are neutralised by antitoxic serums. If, therefore, in spite of there being the same quantity of toxones and of antitoxin in the mixtures, these mixtures become more toxic for the guinea-pig than for the mouse, we have an indication that some special change must take place in the animal to upset the conditions of toxicity.

Weigert[1] accepts the accuracy of Buchner's experiment, which, indeed, can no longer be denied, but explains it on the hypothesis that there is some substance in the animal possessing a very great affinity for the toxin. This substance is supposed to be capable of decomposing the innocuous combination of the antitoxin with the toxin, just as heat does in Calmette's and Wassermann's experiments, described above. In both cases the toxin would be set free to exert its noxious action. Such a hypothesis is very probable, because it agrees with direct observation, but it compels us to accept some new phenomenon which is produced not *in vitro*, but in the living animal, and which carries on its work in a very different fashion in the guinea-pig and in the mouse.

In the present imperfect state of our knowledge it is very difficult to form any idea of the precise conditions which must intervene in the organism of the guinea-pig to cause the tetanus toxin to act in a mixture with antitoxin which is much more innocuous for the mouse. In order, however, to satisfy those who seek to understand these complex phenomena, it may be useful to cite another example of antitoxic action in which certain factors are distinguished by their simplicity.

Lang, Heymans and Masoin[2] have demonstrated that hyposul- [382]
phite of soda prevents poisoning by prussic acid. This terrible poison becomes innocuous if we take care to introduce into the animal by any channel whatever (subcutaneously, intravenously, or by the stomach) a sufficient quantity of hyposulphite of soda. Under these conditions the sulphite is substituted for the hydrogen of the prussic acid, transforming the poison into sulphocyanic acid, which

[1] Lubarsch u. Ostertag's "Ergebnisse d. allgem. Pathologie u. patholog. Anatomie," Wiesbaden, IV Jahrg. (for 1897), S. 121.

[2] *Arch. internat. de Pharmacodyn.*, Gand et Paris, 1896, Vol. III, p. 77.

has no action on the organism. The hyposulphite of soda, then, acts as the antitoxin of the prussic acid, thanks to a chemical reaction of substitution between bodies of simple composition. We have never yet succeeded in reproducing this reaction *in vitro*, whilst in the animal body it is effected with very great ease. Consequently, we are quite justified in invoking special conditions in the body of the living animal; this, however, does not preclude the possibility of a transformation of the toxic substance into an innocuous substance through a chemical reaction. It is probable that analogous phenomena may also be met with in the action of true antitoxins on the microbial toxins or allied substances (venoms, vegetable toxalbumins).

The case of the destruction of micro-organisms, which is now more easily studied because it is possible to observe with the eye the fate of these organisms in the animal, is a further source of valuable information. The direct action of cytases on certain bacteria, such as the cholera vibrio, can be just as easily demonstrated *in vitro* as can the action of antiricin on ricin. If we proceeded to argue from this, a perfectly accurate observation, that the living animal plays no part in the destruction of the micro-organisms and that this destruction takes place always in a fashion analogous to Pfeiffer's phenomenon *in vitro*, we should undoubtedly arrive at an erroneous conclusion. We know already, as has been indicated in previous chapters, that the granular transformation of vibrios is only part of a whole series of phenomena of destruction of micro-organisms, the great majority of which phenomena require more or less active intervention of the animal organism. In reality, matters usually go on in a very complicated fashion, in which direct and indirect actions are blended in varied proportions. In the examples described elsewhere, we see, alongside the granular transformation, an agglutination into
[383] clumps and immobilisation, and an ingestion and intracellular destruction of micro-organisms. The final phase, no doubt, is always a chemical or physico-chemical action, exerted against the micro-organism, but how varied are the means used to bring about this result! We may surely be allowed to suppose that analogous phenomena may take place in the action of antitoxins on the toxins.

Just as, in the analysis of the influence of serums on the micro-organisms, it was found useful to study the action of certain fluids less complicated than the anti-infective specific serums, so we may utilise information furnished by the antitoxic action of fluids other

than the true antitoxins. Cases are by no means rare in which normal serums exert a certain influence on toxins. Thus, Pfeiffer[1] noted that the normal blood serum of the goat has the power to prevent fatal poisoning by the cholera toxin. Freund, Grosz and Jelinek[2] observed an analogous action of solutions of nucleohiston on diphtheria intoxication and Kondratieff[3] demonstrated the same action of an extract of the spleen on the tetanus poison. Calmette[4], in collaboration with Deléarde, studied the influence of a whole series of fluids on abrin intoxication. Whilst physiological saline solution was absolutely incapable of preventing the death of animals, fresh broth exerted an undoubted antitoxic power. Amongst normal serums, ox serum exhibited a certain antirabic property. More, however, than the serums of normal animals, have those of animals immunised against various toxins other than abrin (antitetanus, antidiphtheria, antivenomous serums, &c.) been found to possess the power of preventing intoxication by abrin. These facts are connected with others of analogous nature, previously demonstrated by Calmette[5], of which I may cite the following : the serum of animals vaccinated against tetanus toxin is active, though to a less degree, against snake venom ; the serum of rabbits vaccinated against rabies, a serum powerless to protect against this disease, is, however, very markedly effective against the same venom ; the serum of animals immunised against snake venom is also antitoxic against scorpion venom (I have [384] myself had the opportunity of confirming this fact on several occasions). In all these examples, the serums have proved to be less efficacious against poisons other than the toxin with which the animals that furnished the blood had been treated. Ehrlich[6], too, has demonstrated that animals vaccinated against robin (toxalbumin of *Robinia pseudacacia*) produce a serum, antitoxic not only against this poison but also against ricin. It need scarcely be added that in all these cases of non-specific action of serums derived from vaccinated animals, no question of any antitoxic effect of normal serums can enter. In all the experiments just summarised, the serums of normal animals, used as controls, were found to be inefficacious.

[1] *Ztschr. f. Hyg.*, Leipzig, 1895, Bd. xx, S. 210.

[2] *Centralbl. f. inn. Med.*, Leipzig, 1895, Jahrg. xvi, SS. 913, 937.

[3] *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1896, Bd. xxxvii, S. 191.

[4] *Ann. de l'Inst. Pasteur*, Paris, 1896, t. x, p. 703.

[5] *Ann. de l'Inst. Pasteur*, Paris, 1895, t. ix, p. 225.

[6] "Die Werthbemessung d. Diphtherieheilserums" (*Klin. Jahrb.*, Berlin, 1897, p. 20 of reprint).

If, in the case of the non-specific action of serums, it were allowable to advance the hypothesis of a direct influence of these fluids on the toxins, it would still be impossible to sustain this view where broth fulfils the antitoxic *rôle*. This fluid, much simpler in composition than any serum, is an excellent culture medium for micro-organisms and one in which the toxins develop well and can be kept for a fairly long period. There is, therefore, not the slightest ground for assigning to it any direct antitoxic action, on the contrary, everything leads us to regard it as an indirect agent, which acts by stimulating the reaction of the animal organism. Here, then, the case would be quite analogous to that of the action of broth as a protective agent against certain bacterial injections, a subject already discussed in the tenth chapter. In this same category of indirect influences also, must be ranked the example of the antitoxic action of the blood of the crayfish against scorpion venom. I have demonstrated in a series of experiments that the fresh blood of the crayfish has the power to prevent fatal intoxication of mice by scorpion venom. Injected in a dose of from 1 to 1·25 c.c., several minutes or an hour before the injection of the rapidly fatal dose of scorpion venom, the crayfish's blood exerts a very distinct preventive action. It might be supposed from this that the crayfish belongs to the group of animals insusceptible to scorpion venom. This, however, is not the case. The crayfish is very susceptible to this poison
[385] and succumbs to a quarter the dose necessary to kill a mouse. The blood of the crayfish is, therefore, completely ineffective as a protective to the crayfish itself, and only exerts its action when introduced into the body of the mouse. It might be concluded that it is only after it has been drawn from the crayfish that the blood acquires its antitoxic power. Experiment contradicts this supposition. Crayfish blood, when injected into another crayfish, in equal or greater amount than is necessary to protect a mouse, is incapable of preventing fatal intoxication by scorpion venom, although, here again, the crayfish received only one-quarter of the dose of venom used for the mice.

We are, therefore, compelled to believe that the crayfish's blood is antitoxic for the mouse, not in virtue of its direct neutralising action on the venom, but owing to some indirect influence on the organism of the mouse. It is impossible to define, exactly, the mechanism of this action. We may suppose that the blood of the crayfish contains some substance which, by itself, is insufficient to prevent the intoxication, but which becomes active in the presence of

some other substance, also inefficacious by itself, met with in the organism of the mouse. Here we should have something analogous to what is met with in immunity against micro-organisms where both fixatives and cytases intervene to bring about the destruction of micro-organisms. By making researches *in vitro* on the action of the fluids on bacteria, we may easily observe certain phenomena which appear to indicate their direct influence. Take the case of the fluid of an oedema from an animal vaccinated against the cholera vibrio which renders this micro-organism motionless and agglutinates it *in vitro*; the oedema of an unvaccinated animal produces no such effect. If, however, we were to conclude from this fact that, in the oedema of the living animal or in its subcutaneous tissue, everything goes on as in the test tube and that no other phenomenon of reaction against the vibrios is produced, we should fall into a grave error. It is extremely probable that, in the resistance of the living animal against the toxins, the phenomena are more complicated than are those observed *in vitro*. The example of the blood of the crayfish which prevents the poisoning of the mouse, without having any influence on that of the crayfish itself, may here serve as a guide to us. It is possible that, as in the struggle against the micro-organisms, we have here a co-operation of two substances, each one of which, by itself, is inactive. One of these substances would be found pre- [386]
existent in the blood of the crayfish, the other forming part of the organism of the mouse. Perhaps the action of this blood is even more complicated and only becomes active through the mediation of some constituent of the living cell.

Our study of immunity against toxins long ago revealed cases in which this resistance cannot be attributed simply to the antitoxic action of the body fluids. Animals vaccinated against living micro-organisms may succumb to infection in spite of the presence of a strong anti-infective power of the body fluids; similarly animals immunised against toxins may die from intoxication in spite of the antitoxins contained in their fluids. Facts of this order are not rare. Roux and Vaillard[1] on several occasions observed animals which died from tetanus although they had a large supply of antitoxin in their blood. Von Behring[2] and his collaborators, Knorr, Ransom, and

[1] *Ann. de l'Inst. Pasteur*, Paris, 1893, t. VII, p. 98.

[2] *Deutsche med. Wchnschr.*, Leipzig, 1893, S. 1253; "Allgemeine Therapie der Infectionskrankheiten" in Eulenburg u. Samuel's "Lehrb. d. allg. Therapie," Berlin u. Wien, 1899, Bd. III, S. 1051.

Kitashima, also collected a large number of analogous facts. They showed that horses that have been treated for a long time with tetanus toxin and whose blood serum is very antitoxic, still experience marked illness after fresh injections of toxin and may even succumb, in spite of the presence of a large amount of antitoxin in their blood. In these cases the morbid phenomena are undoubtedly different from those typical of tetanus. Instead of the muscular contractions which characterise this disease, the above observers noted disturbance in the regulation of the body temperature, exudative inflammation around the point of inoculation, impairment of appetite and fall of body weight. Sometimes they observed muscular tremors and marked feebleness in the movements. These symptoms differing from those of typical tetanus, it may be asked whether this poisoning is not due to special substances other than tetanus toxin in the fluids injected. Von Behring does not think that this is the case, for he found that by adding antitetanus serum the formation of exudations at the seat of inoculation was suppressed. These exudations, then, must be attributed to the tetanus toxin.

In the cases where animals immunised against diphtheria toxin fall ill and even die as the result of fresh injections of toxin, in spite
[387] of the presence of a large quantity of antitoxin in their blood, we might also cast doubts on the diphtheritic character of the poisoning, because the clinical picture of this poisoning is not a very typical one. At the Pasteur Institute, where a large supply of antidiphtheria serum is prepared, we see, from time to time, horses, which have long been undergoing the process of immunisation and are furnishing a very good serum, suddenly fall ill and die from intoxication, without presenting any symptom of infective disease. On one occasion, there was actually quite a small epidemic of fatal poisonings as the result of the injection of a quantity of diphtheria toxin not exceeding the doses which had been well borne previously. Amongst the horses, inoculated with the same toxin, five of the best furnishers of serum died. The others, some of which were producing only a weak serum, remained unaffected.

Von Behring and Kitashima[1] have given a detailed history of a young horse which had become very susceptible as the result of vaccination with diphtheria toxin. It finally succumbed to the intoxication in spite of the presence of diphtheria antitoxin in its blood.

If, in these examples, we have any reason to doubt the specific

[1] *Berl. klin. Wchnschr.*, 1901, S. 157.

nature of the intoxication, all doubt must give way before the case described by Brieger[1]. One of his goats, well-immunised with tetanus toxin, which, for months, had furnished a good serum and even an antitetanus milk, after an injection, stronger than the preceding ones, was seized with tetanic contractions. These, becoming general, brought about the death of the animal with the symptoms of classic tetanus. The blood, drawn off after death, exhibited strong antitoxic power.

As the result of these observations von Behring formulated the theory of a hypersusceptibility acquired during immunisation. "Paradoxical as it may appear," he writes[2], "there can no longer exist any doubt that horses which have acquired a high immunity as the result of treatment with tetanus toxin, present a histogenic hypersusceptibility of the organs which react against the tetanus toxin." In support of this thesis von Behring compares the effect produced by this toxin on horses immunised with this same poison and on normal horses treated with antitoxic serum from other horses. The former, in spite of the fact that they contain in their blood 1,500 times [388] more antitoxin than do the latter, are, nevertheless, less refractory to tetanus toxin. This feeble resistance is due, in von Behring's opinion, to the much greater susceptibility of the living elements in the horses treated with repeated doses of the poison.

Von Behring's theory of this form of acquired specific hypersusceptibility has been confirmed by several well-observed facts. These show that, in the animal subjected to treatment by toxins, phenomena of very diverse order are evolved simultaneously: on the one hand, cell reactions which bring about the production of antitoxins; on the other, an increase in the susceptibility of some of the living elements to the specific poison. We are, however, justified in asking if the great difference between the immunity of animals treated with toxin, and that of others treated with antitoxic serum, can be altogether attributed to this hypersusceptibility?

Let us examine in a little more detail some examples of this hypersusceptibility. We know that the guinea-pig is characterised by its great natural susceptibility to the toxins of tetanus and diphtheria. Small doses of these poisons are quite sufficient to produce in it a fatal intoxication. But it is possible to diminish greatly this

[1] *Ztschr. f. Hyg.*, Leipzig, 1895, Bd. XIX, S. 109.

[2] "Allgemeine Therapie der Infectionskrankheiten," in Eulenburg u. Samuel's "Lehrb. d. allg. Therapie," Berlin u. Wien, 1899, Bd. III.

feeble resistance of the guinea-pig by frequent injections of very small quantities of toxin. Knorr[1] increased their susceptibility to tetanus toxin by daily injections of one-tenth of a minimal lethal dose. The animals died before they had received the ten tenths of this dose. The hypersusceptibility produced under these conditions might be so great that one-fiftieth of the minimal lethal dose was capable of causing death. From these facts we can understand the great difficulty experienced in the earlier attempts to vaccinate guinea-pigs by means of unmodified toxin.

Von Behring and Kitashima[2] made analogous researches on the susceptibility of guinea-pigs to diphtheria toxin. By frequent injections of very small doses of this poison they succeeded in killing these animals with $\frac{1}{400}$ of the minimal lethal dose *distributed over several injections.* They never succeeded in vaccinating guinea-pigs with increasing doses of pure diphtheria toxin. Their animals died even when they commenced with one-millionth of the minimal lethal dose.

[389] Here, then, we have examples of the greatest hypersusceptibility that it is possible to observe. When we compare it with the changes in the antitoxic power of the blood, we find that these are even more marked. Thus, Salomonsen and Madsen's horse, to which we have already referred, presented extraordinary oscillations in this power. After receiving, during the course of immunisation, a fresh dose of diphtheria toxin, the antitoxic value of its blood suddenly fell more than one-third (35 °/₀). In order to neutralise, completely, this dose of toxin, when injected into a normal animal mixed with antitoxic serum from this same horse, a very small quantity of the blood of the latter would have been sufficient. The injection into the immunised horse should have passed unperceived, as this animal contained in its body more than 50 litres of strongly antitoxic blood. Nevertheless the antitoxic power of this blood fell 12,000 times more than it ought to have fallen according to the calculation made upon the data just indicated. This fall is incomparably greater than the increase of susceptibility to toxin in the most significant examples reproduced above.

As the fact above cited is not at all unique, it is probable that the phenomena which appear in the animal subjected to vaccination

[1] "Experimentelle Untersuchungen über die Grenzen der Heilungsmöglichkeit des Tetanus," Marburg, 1895, SS. 18, 19.

[2] *Berl. klin. Wchnschr.*, 1901, S. 157.

by toxins, must be much more complicated than is usually supposed. If the fresh injections of these poisons bring about a specific hypersensitiveness on the one hand, and on the other a great fall in antitoxic power, followed by its still more notable augmentation, it is evident that the introduction of toxins must give rise to a great perturbation in the cell functions. The general analogy between acquired immunity against micro-organisms and against toxins probably rests on similar bases. Kretz[1] has already advanced the hypothesis that, in antitoxic action, two factors, comparable to the cytases and fixatives in the antimicrobial action, co-operate. In the absence of one of these elements we can understand that the one which remains may be incapable of bringing about the neutralisation of the toxin. For this reason the antitoxic serum may act very differently in the organism of the animal which produces it and in that of a normal animal which receives it. An explanation which is adequate for the antitoxic action of the blood of the crayfish injected into mice serves equally well in the case of the antitoxic influence [390] of the serums of animals which themselves succumb to intoxication.

Wassermann's[2] experiments on the anticytase serums might appear to supply an argument against the hypothesis we are defending. Having shown that animals injected with antityphoid serum die of intoxication when serum which prevents the action of the cytases is introduced simultaneously, Wassermann put the question: May not the action of the antitoxins be prevented by this same anticytase serum? To solve this point he injected into guinea-pigs a mixture of antidiphtheria serum with toxin in excess and a fairly strong dose (3 c.c.) of anticytase serum, upon which we have already spoken (see Chapter VII). The animals, so treated, behaved exactly as did the animals used for control which received the same quantities of antitoxin and toxin but without the addition of anticytase serum. Wassermann concludes from these experiments that the exclusion of the cytase, contrary to what takes place with antimicrobial serums, in no way impedes the action of the antitoxins. This conclusion, which appears at first sight to be justified, cannot, however, be accepted, as the two examples chosen by Wassermann, typhoid infection and diphtheria intoxication, differ very profoundly from each other. In the former, we have an experimental typhoid peritonitis which kills the control animals in less than 24 hours,

[1] *Ztschr. f. Heilk.*, Berlin, 1901, Bd. XXII, S. 1.
[2] *Ztschr. f. Hyg.*, Leipzig, 1901, Bd. XXXVII, S. 194.

whilst the second is diphtheria in which the controls do not succumb until the sixth day after injection. The effect of the anticytase serum being only very transitory, it is quite natural that this should manifest itself in an infection of short duration and should not do so in a slow intoxication. Besides, Wassermann himself has shown that in several other cases of immunity against micro-organisms (the bacilli of influenza and of leprosy) the injection of his anticytase serums does not interfere with the perfect resistance of the animals. But even were it demonstrated that the cytases really play no part in immunity against toxins, the intervention of some other similar factor could always be evoked.

The analogy between immunity against micro-organisms and that against toxins may facilitate the study of the relations between the latter and the antitoxic power of the body fluids. In the preceding
[391] chapters we have described examples in which animals possess a protective power in their blood but are not refractory to the corresponding infection; on the other hand, we have cited cases in which acquired antimicrobial immunity exists without the blood presenting any appreciable protective power. The idea of measuring acquired immunity against micro-organisms by the measurement of the protective or agglutinative power of the blood must therefore be abandoned, and it is impossible to regard immunity against toxins as a function of the antitoxic property of the body fluids. As we have seen, animals completely refractory to tetanus, such as the cayman, whose immunity does not depend on the antitetanic power of the blood, develop antitoxin after the injection of toxin. A similar state of affairs, but less pronounced, has been demonstrated by Vaillard as occurring in the fowl. The fowl, in spite of its very marked natural immunity against tetanus, produces antitetanin as the result of the introduction into its body of tetanus toxin; the rabbit, on the other hand, a susceptible animal, may acquire a real immunity without the development of any antitoxic power in its fluids. An additional fact was noted by Vaillard[1]. He showed that the repeated inoculation of tetanus spores along with a small quantity of lactic acid, made below the skin of the tail of rabbits procured for them an immunity against tetanus toxin, although no antitoxic property appeared in their blood. In his experiments, one hundred volumes of blood serum were found to be incapable of neutralising a single minimal lethal dose of the toxin. The rabbit, however, still remains

[1] *Compt. rend. Soc. de biol.*, Paris, 1891, p. 464.

quite capable of developing antitetanic power in its fluids. All that is necessary is to inject into it some tetanus toxin heated to 60° C. or treated with Lugol's iodo-ioduretted solution. As the outcome of his researches Vaillard concludes that the antitoxic property of the body fluids "is not sufficient...for the general interpretation of acquired immunity, as it cannot be demonstrated in all animals which have become refractory."

The facts I have just mentioned were demonstrated early in our study of the antitoxic power of the animal organism. Since then a large number of analogous data have been collected. Recently, von Behring and Kitashima[1] have had to abandon the immunisation of monkeys against diphtheria toxin because of the poor yield in anti- [392]
toxin which they obtained. The blood of one of their monkeys that had acquired a resisting power against very large doses of diphtheria toxin showed only a very moderate antitoxic power. In establishments where antitoxic serums are prepared on a large scale the workers have become convinced that the yield of antitoxin has no direct constant ratio to the immunity of the animal. This has been demonstrated repeatedly at the stables of the Pasteur Institute. Thus, of two horses, treated at the same time and in exactly the same way with diphtheria toxin, one furnished a very good antitoxic serum which was maintained at 200 units Ehrlich, rising up to 400 units, whilst the other never reached 150 units[2]. And yet both these animals possessed the same immunity against diphtheria toxin. They tolerate considerable doses of toxin and react merely by a slight or insignificant rise in temperature. In another series of horses, which have been immunised for nearly seven years, one remained capable of yielding a large quantity of antitoxin, seeing that the value of its

[1] *Berlin klin. Wchnschr.*, 1901, S. 157. The idea of immunising monkeys against diphtheria was suggested to von Behring by the fact that the immunity conferred by serums was the more durable the nearer the relation between the serum used and the blood of the species which receives the protective injection. Von Behring supposed that the diphtheria antitoxin, introduced into the human body, would be maintained there longer, if the antitoxic serum injected came from monkeys, species much nearer man than is the horse, the usual source of antidiphtheria serum. The immunity conferred by this horse serum is generally of very short duration.

[2] Ehrlich's antitoxic unit is adopted by most investigators not only in Germany, but also in other countries. This unit corresponds to 1 c.c. of serum capable of neutralising 100 lethal doses of a standard toxin, i.e. that used to establish the first standard of antitoxin. The serum must be injected after being mixed *in vitro* with the toxin. The neutralisation must be complete and give rise to no symptom of intoxication.

serum oscillated between 200 and 300 units. After five years of this state of things the antitoxic power began to fall considerably, without, however, any corresponding loss of immunity. Indeed, an injection of 250 c.c. of toxin (of which 0·002 c.c. was sufficient to kill a guinea-pig) began, at the commencement of the present year, to be borne without the least febrile reaction. An attempt was made to raise the antitoxic power of the blood by making intravenous injections of toxin and of diphtheria culture, but in vain. The yield of antitoxin continued to fall and it became necessary to employ this horse for another purpose than the preparation of antidiphtheria serum. This is by no means an isolated example. Of a large number
[393] of treated horses it frequently happens that certain individuals, without being particularly susceptible to a given toxin, are found to be incapable of producing any corresponding antitoxin[1].

In presence of the fact that animals very resistant to toxins may possess no, or only an insignificant antitoxic power in their fluids, and that, on the other hand, animals in which this property is highly developed may succumb to intoxication, it may be readily understood that immunity against toxins and the antitoxic power of the body fluids may be two distinct conditions. Von Behring has clearly demonstrated the fact of the cellular hypersensitiveness of the animal immunised against the corresponding toxin and has laid great stress upon this fact. He came[2] to the conclusion that "the immunity of the tissues and the production of antitoxin follow a parallel course in their development so slightly that, in spite of an abundant accumulation of antitoxin, the susceptibility of the elements of the tissues may increase in an extraordinary fashion." If, during the course of immunisation, this susceptibility can increase so greatly, it is probable *à priori* that under certain circumstances it might also diminish notably. After demonstrating "that in time the antitoxin disappears from the blood of animals immunised with toxins without any consequent disappearance of immunity," von Behring formulated the conclusion that in these animals "the living elements of the animal, which were previously susceptible to the poisons, have acquired an insusceptibility towards the same substances." This result fully accords with the facts of the change of the negative chemiotaxis of phagocytes into positive

[1] These observations were communicated to me by M. Prevôt, the director of the serotherapeutic station of the Pasteur Institute at Garches.

[2] *Deutsche med. Wchnschr.*, Leipzig, 1893, SS. 1253, 1254.

chemiotaxis for micro-organisms during the acquisition of anti-infective immunity.

Later, von Behring[1] changed his opinion. Whilst still accepting the change of cellular susceptibility in the direction of hypersensitiveness in animals immunised against toxins, he refused to admit the change in the opposite direction. The cells, according to him, never lose any of their susceptibility, so that acquired immunity against toxins cannot be obtained otherwise than by means of [394] antitoxins capable of neutralising the poison in a susceptible or hypersusceptible animal. This new theory von Behring upheld in several papers and it is met with in his most recent publications. Nevertheless, certain well-established facts compel us to accept an immunity against toxins as coming about as the result of a diminution of the susceptibility of the vaccinated animal. Parallel with his researches on the increase of the susceptibility of guinea-pigs to tetanus toxin, researches discussed above, Knorr[2] describes analogous experiments on rabbits. When these animals are injected with fractions of the minimal lethal dose, frequently repeated, the rabbit not only does not become hypersusceptible to tetanus but exhibits a greater and greater insusceptibility. Whilst guinea-pigs, treated according to this method, die from tetanus before they have reached the minimal lethal dose, rabbits, as the result of frequent injections of small quantities of tetanus toxin, become capable of resisting five times the lethal dose (for normal rabbits) without exhibiting the slightest symptom of illness. Against the attribution of this result to the acquired insusceptibility of the living animals it might be objected that the immunity, in this case, may depend on the antitoxic power of the fluids of the body, developed with great rapidity. Such an objection cannot be raised in the case of horses which become insusceptible to toxins after a long period of vaccination. The horse whose history was given above, when discussing the diminution of antitoxic power, may serve as an example. At the commencement of its vaccinal period, in 1894, it reacted to the injection of 10 c.c. of diphtheria toxin by a rise of temperature of 1° C. Four years later, when its blood had become very antitoxic (350 units per c.c.), it was necessary to inject 350 c.c.

[1] Article "Immunität" in Eulenburg's *Realencyclopädie*, III^te^ Aufl., Wien, 1896; see also his "Allgemeine Therapie d. Infectionskrankheiten," in Eulenburg u. Samuel's "Lehrb. d. allg. Therapie," Berlin u. Wien, 1899, Bd. III, SS. 996, 997.

[2] *Op. cit. supra* p. 370, S. 19.

of toxin to obtain the same rise of temperature. Quite recently, having now lost the greater part of its humoral antitoxic power, this horse exhibited no rise of temperature after an injection of 250 c.c. of strong diphtheria toxin. The diminution of the specific susceptibility is produced in this case in a most marked fashion; it is not therefore to the antitoxic property of the body fluids that this case of immunity must be attributed.

The insusceptibility acquired against poisons of different kinds is observed also in cases where the adaptation is not accompanied
[395] by the production of humoral antitoxic properties, as in the immunity of frogs against abrin. This form of immunity may be traced through the organic series down to such lowly developed organisms as the plasmodium of the Myxomycetes, which as we have seen readily becomes adapted to different poisons (see Chapter II).

It can be clearly seen, then, that immunity against toxic substances is a very complex phenomenon which it is impossible to reduce simply to an antitoxic function of the fluids of the body. For this reason we cannot accept a theory which would confine this kind of immunity within the narrow limits of a simple reaction between two substances, a reaction quite comparable to that observed in a test-tube. Attempts have been made to determine with almost mathematical precision the conditions under which it is possible to communicate to the animal a resistance against microbial toxins and formulae have been constructed to define these conditions. But the application of these formulae has been found to be a much more difficult matter. In Prussia, with the sanction of the Government, regulations have been enacted as to the procedure to be followed in the testing of antitoxic serums, and a paragraph has been added which requires a post-mortem examination of the guinea-pigs employed for this purpose in the case of diphtheria antitoxin. "The dead animals," says this instruction, "must be submitted to a post-mortem examination, and special attention must be directed to the presence of any pre-existing diseases (tuberculosis, pseudotuberculosis, pneumonia) which may have induced hypersusceptibility in the animals under experiment." Do we not see in this a proof of the important intervention of the organism of the living animal which may modify the results of calculations based upon too rigorous formulae? It must not be forgotten, too, that in addition to the three diseases named in the instructions, we have a number of other factors which may influence the receptivity and the resistance of

animals. We have already cited Roux and Vaillard's experiments which demonstrated that even animals which have been previously subjected to vaccinal inoculations against certain micro-organisms, exhibit a hypersusceptibility to mixtures of toxins with antitoxins.

In view, then, of this complexity of the phenomena of acquired immunity against toxins, it would be very important could we learn something of the nature and origin of antitoxins. Unfortunately, as we shall see, these questions are, as yet, far from having received a satisfactory solution.

Struck by the fact that antitoxins exert a specific action on the toxin which has been employed in the treatment of the animals that produce the serum, certain observers have sought an explanation on [396] the hypothesis of a transformation of toxin into antitoxin. We have already seen that antitoxic action is not always absolutely specific; we have serums which prevent intoxication by various kinds of poisons, e.g. antitetanus serum, which is active against both tetanus toxin and snake venom. There is, however, a great quantitative difference between the influence of the antitoxin on the toxin with which the animals have been prepared and on a different poison. Thus, in the example just cited, in order to neutralise snake venom it is necessary to use a much larger quantity of antitetanus serum than against the toxin of tetanus. The classical example of the specific influence of antitoxins is the absolute inactivity of antidiphtheria serum against tetanus and the same non-effect of antitetanus serum against diphtheria intoxication. The most simple explanation of this specificity of action appeared to be the supposition that each antitoxin contains a part of the corresponding toxin, modified by the organism of the animal. H. Buchner[1] advocates this hypothesis. I myself[2] said "that it is probable that antitoxins, at least in great part, represent a modification of the toxins prepared by certain cells in the animal body; this product is then poured into the blood." This view was stated as a "probability" and consequently contains no affirmation in the least definitive. I was, therefore, quite prepared to give it up under the weight of the crushing criticism formulated by several very distinguished observers. It was objected; first, that antitoxin is produced by animals in very great disproportion to the quantity of toxin they have received; secondly, that the animals which receive

[1] *München. med. Wchnschr.*, 1893, S. 380.

[2] "Immunität" in Weyl's "Handbuch der Hygiene," Jena, 1897, Bd. IX, S. 48.

an injection of antitoxin eliminate it from their body much more rapidly than do those which prepare it in their own body; thirdly, that antitoxins are sometimes found in the blood of healthy animals, who have had no attack of the disease nor any injection of the specific toxin. Let us examine these objections more closely, objections all based on well-established facts.

It has been shown that the antitoxin produced by the animal is sufficient to neutralise a quantity of toxin much greater than that which was injected into the animals supplying the antitoxic serum.
[397] Knorr[1], from his experiments, calculated that a horse reacts to one unit of toxin by the production of 100,000 units of antitoxin. This statement certainly does not allow us to affirm that all the antitoxin corresponds to toxin, but it does not eliminate the possibility that toxin, subjected to the influence of the cells of the animal body, may be found, in a modified form, in the product of these elements. This hypothesis would be quite sufficient to explain the very remarkable specificity of antitoxins.

If the toxin, in order to be modified by the living cells, must be subjected to some special action on the part of the latter, we can readily understand that this process must demand a greater or less length of time; this would lead to a much slower elimination of the antitoxin than in the case where it had been injected, ready prepared, into a normal animal. From the commencement of his researches on immunity against poisons, Ehrlich[2] distinguishes two kinds of this immunity, an *active immunity* which is obtained as the result of the introduction of toxins into the animal, and a *passive immunity*, another form of the refractory condition which is set up by the injection of antitoxic serum formed in the actively immunised animal. Von Behring[3] applies the term *isopathic immunity* to active immunity, and to passive immunity that of *antitoxic immunity*. It is generally admitted that the first kind of immunity is more slowly acquired, but that it persists for a much longer period than the second (passive or antitoxic immunity) which is acquired immediately after the introduction of the antitoxin, but which, on the other hand, lasts for a short time only. This view is supported by numerous observations on the very rapid disappearance of the

[1] *München. med. Wchnschr.*, 1898, p. 321.

[2] *Deutsche med. Wchnschr.*, Leipzig, 1891, SS. 976, 1218; [*Ztschr. f. Hyg.*, Leipzig, 1892, Bd. XII, S. 183].

[3] "Allgemeine Therapie der Infectionskrankheiten" in Eulenburg u. Samuel's "Lehrbuch der allgemeine Therapie," Berlin u. Wien, 1899, Bd. III, S. 997.

refractory condition. According to von Behring the great difference in the duration of the isopathic and antitoxic immunities is only an apparent one. It is due to the fact that antitoxins are usually introduced along with the serum of different species which sets up a strong reaction and is rapidly eliminated from the animal. Thus the antitoxic serum of the horse is usually injected into small animals such as guinea-pigs, rabbits, and mice. When, however, von Behring injected horses with antitoxic serums from other horses, [398] the antitoxic immunity lasted almost as long as in animals vaccinated with toxins. Ransom[1] has developed this thesis in a work carried out in von Behring's Institute at Marburg, and supports it by comparative researches which demonstrate the more rapid disappearance of the antitoxin when introduced with the serum of a different species than when introduced with that of the same species.

Even should we accept the current view on the greater duration of the antitoxic power of the blood in isopathic immunity, the hypothesis of the transformation of toxin by the cells of the animal is not necessarily invalidated. If a part of the toxin introduced into the animal remains stored for some time in an organ it is evident that only gradually can it be subjected to the transforming action of the cells. It is impossible, in the present state of our knowledge, to demonstrate this proposition, but we may invoke in its favour the prolonged persistence of red blood corpuscles when introduced into the body of a different species of animal (see Chapter IV). These corpuscles are in the end always completely digested but the process is of long duration.

The same hypothesis will also explain a fact, first demonstrated by Roux and Vaillard[2]. They have shown that after repeated bleedings of rabbits immunised against tetanus, the antitoxic property of the blood was soon raised to almost the same value as before. Salomonsen and Madsen[3] have confirmed the fact of the regeneration of antitoxin after the bleeding of their animals (horses and goats) immunised against diphtheria. Those authors who do not accept the possibility of the transformation of toxins in the production of antitoxins, regard these facts as absolutely incompatible with the hypothesis which they attack. Thus, Weigert[4] considers that the

[1] *Journ. Path. and Bacteriol.*, Edin. and London, 1900, Vol. VI, p. 180.
[2] *Ann. de l'Inst. Pasteur*, Paris, 1893, t. VII, p. 82.
[3] *Ann. de l'Inst. Pasteur*, Paris, 1898, t. XII, p. 763.
[4] *Op. cit. supra*, p. 363, IV Jahrg., S. 122.

regeneration of antitoxin after bleeding can only be understood by accepting that antitoxin, like the blood, may be reproduced in the actively immunised animal without any fresh introduction of toxin. It is, however, just as simple, we think, to explain the fact in question by the hypothesis of a provision of toxin stored up in
[399] certain cells. This also is sufficient explanation of another observation made by Salomonsen and Madsen[1], who showed that pilocarpin is capable of augmenting the production of antitoxin. Since it is the living cells which transform the toxin and excrete the antitoxin, it is quite natural to suppose that every factor which stimulates cell function may be capable of causing an increase of the product transformed by the cells.

The third argument invoked against the possibility of the transformation of toxins into antitoxins is based on the fact that the serum of normal horses has sometimes a certain degree of antitoxic power against diphtheria toxin. The horses have never suffered from diphtheria, therefore the antidiphtherin of their blood has nothing to do with diphtheria toxin. It is not known why the blood serum of certain untreated horses is from the first active against diphtheria toxin, whilst that of others exerts absolutely no action on the same poison. We know only that this property is far from being constant in the equine species. Perhaps it is acquired as the result of the penetration into the animal of some pseudo-diphtheria bacillus, whose frequency and number are very great. In order that the microbial products may give rise to the formation of antibodies, it is not at all necessary that the micro-organisms should produce an evident disease. Thus, to cite one example only, Foerster[2] observed a considerable agglutinative power against the typhoid cocco bacillus in the serum of a child which was found living among a family of typhoid patients but which, itself, presented no morbid symptom.

The criticism, directed against the hypothesis that modified toxin enters into the production of antitoxin, may not be sufficient to show the incorrectness of this view; it does not follow, however, that the view is right. In the present state of our knowledge it is impossible to solve the problem definitely, and as the hypothesis of transformation gives us the best idea of the specificity of the action of antitoxins, it has a right to be taken into consideration as much as any other.

[1] *Compt. rend. Acad. d. sc.*, Paris, 1898, t. CXXVI, p. 1229.
[2] *Ztschr. f. Hyg.*, Leipzig, 1897, Bd. XXIV, S. 514.

Ehrlich[1] has formulated another hypothesis to explain not only this specificity but the origin of antitoxins in general. This is the [400] ingenious hypothesis of side-chains or of receptors, which has already been considered in other chapters of this work. It is now for the first time brought forward in relation to the antitoxins properly so-called, that is to say substances capable of preventing intoxication by microbial toxins. In order to make his hypothesis as clear as possible Ehrlich begins by explaining its bearing on the concrete example of tetanus antitoxin. "When we introduce into an animal a small quantity of tetanus toxin, it is easy to obtain exact proof that it is quickly fixed by the central nervous system, probably by the motor cells of the ganglia; that the central nervous system more than any other organ attracts the tetanus toxin and retains its toxic molecules very firmly." There we have the side-chains of the protoplasm fulfilling this rôle and subjecting the living protoplasm to the prolonged action of the poison. Once it is combined, the side-chain becomes incapable of fulfilling its normal function, and there is induced on the part of the living elements the production of new chains of a similar character. Following the law that the reaction is stronger than the action, there is an over-production of these side-chains which finally so embarrass the cell which has developed them that they are excreted by it into the blood plasma. Once expelled into this plasma, they continue to manifest their affinity for the tetanus toxin, an affinity which must be even greater in the case where the chains are found in the blood than when they were connected with the cell. Owing to this affinity, these chains, now in the blood, fix the tetanus poison introduced into the animal and prevent it from reaching the susceptible nerve elements. Antitoxins, according to this hypothesis are, therefore, nothing but overplus side-chains poured into the body fluids. Ehrlich extends his theory to a whole series of bodies capable of causing the formation of antitoxins and antidiastases. "It is probable," he says, "that all analogous bodies can only become toxic to the animal on condition that the animal is capable of fixing their toxophore groups in certain of the organs that are important for its life" (p. 17).

According to this theory tetanus antitoxin must pre-exist in the central nervous system of the normal animal. In the immunised animal, the side-chains must be reproduced in very great quantity

[1] "Die Werthbemessung des Diphtherieheilserums" (*Klin. Jahrb.*, Berlin, 1897, Bd. VI), SS. 13—17 of reprint.

[401] in the nerve cells and pass thence into the circulation. Indeed, Wassermann, a supporter of this theory, made a search for tetanus antitoxin in the nerve centres of normal animals. In collaboration with Takaki[1] he made the important discovery that the brain and spinal cord of small mammals (guinea-pigs and rabbits) when triturated with tetanus toxin prevent the manifestation of its toxic action in animals most susceptible to tetanus. The brain was always found to be more active than the spinal cord. The property of neutralising the toxin of tetanus belongs to the solid parts of the nerve centres; the fluid of the cerebral emulsion is incapable of exercising this action.

This discovery was soon confirmed. Ransom[2] demonstrated it almost at the same time, and independently of Wassermann and Takaki; and the fact is indisputable. It remains to be seen whether the "antitoxin" of the nerve centres of normal animals is really the same as that which is found in the fluids of animals immunised against tetanus toxin, as is accepted by Wassermann and the other partisans of the side-chain theory. The former is characterised by a very local reaction; it is incapable of being dissolved and distributed through the body of the animal. This is shown by Marie's[3] experiments, and my own[4], all carried out in my laboratory. All that is necessary is to introduce, beneath the dorsal surface of the thigh of a guinea-pig, a quantity of the cerebral substance sufficient to neutralise several times the lethal dose of toxin, and below the skin of the ventral aspect of the same thigh, a lethal dose of this toxin, when it will be found that the guinea-pig contracts a fatal tetanus. The antitoxic action of the nerve substance extends, therefore, for a short distance only; it is strictly local.

The view that the action of the substance of the pounded nerve centres is different from the neutralisation of the toxin by the antitoxin of the body fluids is further confirmed by the fact that the fixation of the tetanus poison by the cerebral substance is very transient. We have shown that a mixture of toxin and pounded cerebral substance, that does not produce any tetanic symptom when injected into the peritoneal cavity of guinea-pigs, sets up a grave tetanus when it is injected subcutaneously into the thigh. In the

[1] *Berl. klin. Wchnschr.*, 1898, S. 5.
[2] *Deutsche med. Wchnschr.*, Leipzig, 1898, S. 68.
[3] *Ann. de l'Inst. Pasteur*, Paris, 1898, t. XII, p. 91.
[4] *Ann. de l'Inst. Pasteur*, Paris, 1898, t. XII, pp. 81, 263.

latter case the toxin becomes separated from the particles of the [402]
cerebral substance that had fixed it. Danysz[1] convinced himself that the mixture of pounded brain with tetanus toxin when it is left in physiological saline solution, in distilled water, or in a 10 °/₀ solution of sea salt, allows the tetanus toxin to pass into the macerating fluid. The fixation of the toxin to the cerebral substance is, therefore, more comparable to the mordanting of colouring-matters by the tissues than to a real combination.

Observers who have repeated the experiments of Wassermann and Takaki have been greatly struck by the difference between the action of the pounded cerebral substance and that of the living brain upon the tetanus toxin. Whereas the former, taken from the guinea-pig, an animal very susceptible to tetanus, prevented intoxication when employed in minimal dose, the living brain of the same species was found to be incapable of neutralising the most minute quantities of toxin. On the other hand, Roux and Borrel[2] have shown that the brain of rabbits, whether untreated or vaccinated against tetanus, was very susceptible to the action of the tetanus toxin. This toxin, injected directly into the brain, set up in both groups of rabbits a special and characteristic cerebral tetanus. On the other hand, when a little of the cerebral substance of the rabbits, mixed *in vitro* with tetanus toxin, was injected into other susceptible animals, these remained unaffected.

This great difference between the antitoxic action of the living brain and that of the pounded cerebral matter, on the one hand, and the rigorous localisation of the antitetanic influence of this cerebral substance, on the other, have suggested to several observers the idea that the brain cannot be regarded as the organ of formation of the true antitoxin, such as is found in the fluids of immunised animals. This view has been expressed by Roux and Borrel, Marie and ourselves. Knorr[3] also shares this view, being struck by the fact that rabbits attacked by tetanus remain for weeks with contractions, but are incapable of producing in their nerve-cells sufficient antitoxin to disintoxicate them, although their blood is already loaded with dissolved antitoxin.

At this period it was generally supposed that, in accordance with [403]
Ehrlich's theory, the hypothetical side-chains were capable, under

[1] *Ann. de l'Inst. Pasteur*, Paris, 1899, t. XIII, p. 156.
[2] *Ann. de l'Inst. Pasteur*, Paris, 1898, t. XII, p. 225.
[3] *München. med. Wchnschr.*, 1898.

certain conditions, not only of fixing the tetanus toxin, but also of neutralising it. It was said, therefore, that these chains, reproduced in large quantities in the cerebral cells, must exercise their neutralising action in the brain itself. Consequently, when it was seen that, in Roux and Borrel's experiments on vaccinated rabbits, this organ was itself affected, it was concluded that the brain must not be regarded as the producer of the antitoxin.

Later, Ehrlich and his supporters, amongst whom I will name especially Weigert, have developed the theory of side-chains in a much more detailed fashion, leading to a different interpretation of several facts previously established. Ehrlich distinguishes in the toxin molecule a *haptophore group* which combines with the side-chain or the corresponding receptor of the living elements, and a *toxophore group* which produces the poisoning of the protoplasm. The side-chains, inactive for the toxophore group, neutralise only the haptophore group. Consequently, when these side-chains are numerous in the nerve elements which produce them, they may be a source of great danger to this living element, by attracting the toxic molecules. In this case, these chains, or receptors, serve to attract the poison, just as the badly adjusted lightning conductor attracts lightning. For this reason rabbits vaccinated against tetanus become tetanic when the toxin is injected directly into the brain. It is only at a distance from the nerve centres that the receptors, excreted into the body fluids, fulfil their rôle of true antitoxins. There they combine with the haptophore group of the toxic molecule, leaving the toxophore group intact; this latter group, however, diverted from the nerve-cells, is incapable of exercising an injurious action.

From this point of view not only the cerebral tetanus of vaccinated rabbits, but also the hypersusceptibility of immunised animals, upon which von Behring has so strongly insisted, may be explained. The argument, drawn from these facts, against the nervous origin of tetanus antitoxin, loses, therefore, much of its weight. If we confront this hypothesis with the other data collected on the question, the solution of the problem becomes beset with great difficulties. Previous
[404] to the discovery made by Wassermann and Takaki, I attempted to solve the problem by removing from fowls portions of the brain and spinal cord, proposing to take advantage of the fact that birds, which are capable of producing antitoxins, withstand these operations fairly well. My hopes were not fulfilled; I could never keep my fowls

alive long enough to complete the experiment. We must, therefore, for the present, be content with indirect arguments. If the nerve centres do really produce the tetanus antitoxin and excrete it into the blood, we ought at a given moment to find in these organs a greater quantity of this substance than in the blood and the other organs. The reader will recall the researches of Pfeiffer and Marx, and of Deutsch, who demonstrated the possession of a greater richness in protective substance by the phagocytic organs of animals, treated with micro-organisms, than by the blood serum. The same result might be obtained by a comparative investigation of the tetanus antitoxin in the nerve centres and the blood of animals immunised against tetanus. My experiments directed to this point have not been favourable to the hypothesis of the nervous origin of tetanus antitoxin.

In fowls, killed as soon as tetanus antitoxin began to appear in the blood, the brain and spinal cord did not exhibit the slightest antitoxic power[1]. We might be tempted to explain this result as due to an accumulation of toxin in the nerve centres which would prevent the manifestation of the antitoxin. For this reason, in my later researches[2], I made use of animals that had been long immunised, but whose blood was still antitoxic. I killed a fowl which had not received any toxin for about eight months, and a guinea-pig into which the last toxic injection had been made almost two years before the date of this experiment. After removing a portion of the brain the blood of these two animals was found to be more antitoxic than before the operation, which indicated that the source of the antitoxin was as yet uninjured. To ascertain whether this source was to be found in the nerve centres I made a comparative determination of the antitoxic power of the brain, of the spinal cord and also of several other organs, of the blood and of the exudations. The result was still negative. The nerve centres were found to be less antitoxic than the blood and other fluids of the body, and even less active than such organs as the liver and kidneys.

In support of the hypothesis of the nervous origin of tetanus [405] antitoxin there remains, then, only the fact of the retarding action of the cerebral substance upon tetanus. In the absence of other arguments this assumes a preponderating importance. We have seen that this action is based on a fleeting and not very firm fixation of the

[1] *Ann. de l'Inst. Pasteur*, Paris, 1897, t. xi, p. 801.
[2] *Ann. de l'Inst. Pasteur*, Paris, 1898, t. xii, p. 81.

toxin by certain parts of the brain and the cord. Are we justified in regarding this as comparable to the more stable fixation observed in living animals susceptible to tetanus intoxication? Soon after Wassermann and Takaki's discovery I pointed out that the pounded brain of frogs mixed with tetanus toxin does not prevent animals, into which this mixture is injected, from contracting fatal tetanus. This observation was confirmed by Courmont and Doyon[1], in several series of experiments carried out under various conditions. They found that "the brain of the frog, heated or unheated, when mixed with tetanus toxin even for several hours, at the temperature of the laboratory or at 38° C., even in considerable doses, does not possess any neutralising property." This fact would not be in any way wonderful if we had to do with an animal insusceptible to tetanus; but in the frog, as we have said in the preceding chapter, this is far from being the case. In the cold it does not readily become tetanic, but above 25°—30° C. it becomes very susceptible. The tortoise, which is very refractory to this intoxication, has a brain which, when pounded and mixed with tetanus toxin, exerts a certain preventive power over susceptible animals. Nevertheless, the brain of the living frog, as demonstrated by Morgenroth, absorbs this toxin. There is, therefore, a difference between the absorption of the tetanus poison by the living elements and by the pounded cerebral substance. A similar result is obtained with several other toxins. Diphtheria poison is very toxic when injected directly into the brain of the guinea-pig or rabbit. Even the rat, as demonstrated by Roux and Borrel[2], is readily affected by this toxin under these conditions. Doses which when inoculated subcutaneously are well borne by the rat, when introduced into the brain set up a fatal intoxication in this animal. And yet the
[406] brain, when pounded and mixed with diphtheria toxin, can never protect susceptible animals from intoxication. Numerous attempts to reproduce Wassermann and Takaki's experiment with the diphtheria poison have always been unsuccessful. Attempts to obtain the same result with snake venom have also given negative results. Calmette[3] made several experiments with emulsions of rabbit's brain and snake venom with the object of ascertaining whether the elements of the nervous system possess against venom the same properties as against tetanus toxin. "None of these emulsions"—concludes Calmette—"exhibited either the slightest protective or antitoxic power *in vitro*.

[1] *Compt. rend. Soc. de biol.*, Paris, 1898, p. 602.
[2] *Ann. de l'Inst. Pasteur*, Paris, 1898, t. XII, p. 238.
[3] *l.c.* p. 343.

There is, therefore, no analogy of action between what takes place in the nerve elements against tetanus toxin and against venom." Nevertheless venom, like diphtheria toxin and tetanus toxin in the frog, exerts an undoubted action on the nerve centres.

Again, the protective fixation of poisons to the cerebral substance is not the exclusive privilege of tetanus toxin. Kempner and Schepilewsky[1] obtained the same result with the toxin of botulism (produced by van Ermenghem's anaerobic micro-organism which sets up intoxication of intestinal origin in certain cases of poisoning by food). The brain and spinal cord of the guinea-pig, when triturated with physiological salt solution and mixed with botulinic toxin, prevents intoxication in susceptible animals, exactly as in Wassermann and Takaki's experiments with tetanus.

When Kempner and Schepilewsky wished to obtain some idea as to the substance or substances in the nerve centres which fix the toxin of botulism and thus prevent poisoning, they found that lecithin and cholesterin, mixed with this toxin or injected separately and simultaneously, protected mice just as completely as did the cerebral substance. On the other hand, they found a difference as regards the two substances when injected before the toxin was introduced; they were then unable to prevent poisoning, though the cerebral substance exerted an undoubted protective influence. Kempner and Schepilewsky also showed that heating altered the preventive action of lecithin and cholesterin less than it did that of cerebral emulsion.

These observers extended their researches to the protective action [407]
of fats and demonstrated that olive oil when emulsified and neutralised with soda and mixed with twice and even four times the lethal dose of botulinic toxin, prevented the contraction of a fatal poisoning by mice. Tyrosin also protected mice against this intoxication, not only when injected simultaneously with the poison, but even when introduced into the animal 24 hours before the poison was administered. Kempner and Schepilewsky conclude "that not only with the substance of the nerve centres, but also with various other substances, they were able to obtain a certain protective effect against the toxin of botulism" (p. 221). Their experiments with cholesterin and tyrosin were suggested to them by the previous researches of Phisalix[2] who demonstrated that the bile salts, as well as the two

[1] *Ztschr. f. Hyg.*, Leipzig, 1898, Bd. XXVII, S. 213.

[2] *Compt. rend. Acad. d. sc.*, Paris, 1897, p. 1053; and 1898, p. 431; *Compt. rend. Soc. de biol.*, Paris, 1897, p. 1057; and 1898, p. 153.

substances I have just mentioned, would protect animals against the venom of the viper.

Bearing all these facts in mind, it appears to be probable that in the above cases it is principally the fatty matters of the nerve centres that temporarily fix these toxins, and allow the animal organism to divert the poisons from their morbific action. From this point of view, it is interesting to note that the toxic action of the tetanus poison can also be prevented by other substances than the emulsion of the nerve centres. Thus Stoudensky[1] demonstrated, in an investigation carried out in Roux's laboratory, that carmine fixes the tetanus toxin and prevents its action on the guinea-pig. As in the case of the cerebral substance, this fixation by carmine is very unstable. When the carmine that has fixed the tetanotoxin is macerated in distilled water it gives up the poison to the water which is then capable of producing tetanus. Such fixation does not end, any more than in the case of the cerebral substance, in the destruction or disappearance of the toxin. Carmine if first dissolved or macerated in water (especially if heated) loses its fixative power and can no longer prevent tetanus poisoning. Sterilisation, at 120°, 100° and even at 60° C., of the carmine, suspended in physiological salt solution, caused it to lose its protective action, although dry heat applied to it in closed tubes did not destroy this power.

[408] In many respects carmine, which is derived especially from the adipose body of the cochineal insect, exerts an antitoxic influence analogous to that of maceration with the nerve centres. If fats play a special part in this action, we can readily understand how a brain, such as that of the frog, poor in fatty matters, cannot fix the tetanus toxin and prevent its morbific action. In any case the fact that certain substances of diverse nature, acting on toxins, exert an influence similar to that of the pounded mass of the nerve centres, does not allow us to accept Wassermann and Takaki's experiment as proving the nervous origin of tetanus antitoxin. The analogy with the facts bearing on the anticytotoxins, collected and described in the fifth chapter, also tells against this hypothesis. We would here remind the reader that the two constituent parts of the antispermotoxin, the anticytase and the antispermofixative, develop in castrated animals and are consequently produced outside the spermatozoa, elements susceptible to the spermotoxin. The facts collected con-

[1] *Ann. de l'Inst. Pasteur*, Paris, 1899, t. XIII, p. 126.

cerning the antihaemotoxins indicate also that these substances have some other origin than the red blood corpuscles.

This latter supposition appears to be in contradiction to Ransom's[1] very interesting researches on the haemolytic action of saponin, carried out in Meyer's laboratory at Marburg. This glucoside, owing to its property of fixing itself on the stroma of these corpuscles dissolves the red corpuscles of many vertebrates. The cholesterin of this stroma combines with the saponin, as the result of which the red corpuscles become altered and allow the haemoglobin to diffuse. But this same substance, cholesterin, which causes the poison to penetrate into the red blood corpuscles, prevents the solution of these elements when they are bathed in blood-serum. This fluid, in fact, acts as the antitoxin to saponin and does so just because it contains cholesterin. The cholesterin of the serum, fixing the saponin, prevents it from affecting the red corpuscles, thus fulfilling the function of a well fitted lightning conductor. On the other hand, when the cholesterin of the stroma of these corpuscles is linked on to the saponin, it renders them the disservice of a defective lightning conductor. The accord between these facts and the postulates of Ehrlich's theory led Ransom to suppose that in the haemolysins and antihaemolysins, [409] cholesterin perhaps played a similar part. His experiments convinced him that this was not the case. As it is generally accepted, after Calmette's[2] experiments and according to Ehrlich's view, that the alkaloids and the glucosides in general are incapable of setting up the formation of antitoxins, we might regard the attempts to find an antisaponin and to settle whether it is identical with cholesterin as useless. But in regard to these delicate questions we must be careful not to give too great weight to *a priori* arguments. It was believed until quite recently that substances with very complex molecules, such as the albuminoids, toxins and soluble ferments, must always give rise to the production of antibodies in the animal; whilst the simpler substances whose chemical nature was better defined could never lead to this. Facts acquired in recent years have led to a modification of this view. In our fifth chapter we have already spoken of the fruitless attempts of Ehrlich and Morgenroth to obtain certain antifixatives. And yet the fixatives, as is shown by the results of the researches of Bordet and myself, belong to the category of substances which are quite capable of setting up the formation of

[1] *Deutsche med. Wchnschr.*, Leipzig, 1901, S. 194.
[2] *Ann. de l'Inst. Pasteur*, Paris, 1895, t. IX, p. 244.

antibodies. Again, certain mineral poisons, quite unexpectedly, gave rise to the development of the counter-poison in the animal body. This fact forced itself upon Besredka[1] in his researches on the adaptation to arsenic made in my laboratory. His experiments were undertaken for the purpose of studying the mechanism of the refractory condition against a poison, apart from any antitoxic action whatever, which, according to previous investigations, seemed excluded. This action, however, was exhibited in such a degree that it could not be ignored. The serum of animals immunised against arsenious acid was found to possess both protective and antitoxic properties against a dose of this poison killing a rabbit in 48 hours. It is true that Morishima[2], in a research carried out in Heyman's laboratory at Ghent, has thrown doubt upon these results. His objections, how-
[410] ever, cannot refute the statements of Besredka which rest on very precise and numerous experiments which I witnessed. Morishima left out of account several important circumstances and carried out his experiments without any continuous check by means of control animals. It must be said also that the resistance of the rabbit against arsenic depends on many different factors and that, at certain seasons, it is much more difficult to adapt them to the poison than at others. It is only by numerous researches extending over a very long period that we can arrive at precise and conclusive results.

From these observations there is every inducement for us to attempt to ascertain whether, by subjecting animals to repeated injections of saponin, it is possible to augment the antisaponic power of their blood-serum and whether, if this takes place, the antitoxic action is due to a rise in the amount of cholesterin in this serum. I therefore requested Besredka to carry out some experiments bearing on this point. Guinea-pigs, injected with progressive doses of saponin for more than two months, at the end of this period showed no increase in the antisaponic power of their serum. They followed the rule established by Ehrlich ; they developed no antitoxin against a glucoside. Moreover, they gave us no new information as to the origin of these antibodies.

In his first memoir in which the theory of side-chains is treated, Ehrlich insists on the nervous origin of antitetanin as an example of the production of antitoxins by animals susceptible to poisons. Now, however, that he has come to distinguish haptophore and toxophore

[1] *Ann. de l'Inst. Pasteur*, Paris, 1899, t. XIII, p. 465.
[2] *Arch. internat. de Pharmacodyn.*, Gand et Paris, 1900, vol. VII, p. 65.

groups in the toxic molecule, it is to the side-chain, which fixes the first group, that Ehrlich attributes prime importance. "The formation of antitoxins"—he says[1] in the opening address at his Institute at Frankfort—"would, therefore, be absolutely independent of the action of the toxophore elements." In other words, for a cell to be capable of producing antitoxin, it is not at all necessary that it should be susceptible to the toxic influence of the poison; it is only necessary that it should possess receptors, or side-chains, capable of combining with the haptophore group of the toxin. Thus it is possible, as we have described above, to produce antitoxins, with modified toxins [411] whose toxic action is *nil* or almost so, but which have retained their power of combining with antitoxic substances. According to Ehrlich, these modified toxins are *toxoids*, in which the toxophore group is completely destroyed; "whilst the haptophore group, the producer of immunising substances, is retained in its integrity." It is evident then that, under such conditions, the tetanus antitoxin might be developed elsewhere than in the nerve centres. For that it would be sufficient that outside the nerve cells there should be other living elements capable of fixing the tetanus toxin, or, to use Ehrlich's phraseology, elements, possessing side-chains, having an affinity for the haptophore group of the tetanus poison.

Dönitz[2] has already expressed the view that in the rabbit the tetanus toxin may be fixed not only by the nerve elements but also by the various other cells.

The existence of such cells, outside the nervous system, is not merely hypothetical. It is shown very clearly in Roux and Borrel's experiments on cerebral tetanus. In order to produce this disease in the rabbit, it is sufficient to introduce a very small dose of toxin directly into the brain. When inoculated subcutaneously with much larger quantities of the same tetanus poison, the rabbit remains in good health or exhibits merely a slight and transient tetanus. "The resistance of the rabbit against the tetanus toxin, injected under the usual conditions"—conclude Roux and Borrel[3]—"is not due, then, to a relative insusceptibility of the nerve centres, but to the fact that much of the poison introduced does not reach the nerve cells and is destroyed in some part of the animal." In the guinea-pig, as shown by the same investigators, the difference of the dose of tetanus poison, necessary to produce fatal tetanus by intracerebral or

[1] *Semaine méd.*, Paris, 1899, p. 411.

[2] *Deutsche med. Wchnschr.*, Leipzig, 1897, S. 428.

[3] *l.c.* p. 229.

by subcutaneous injection, is minimal or *nil*, from which it may be argued that in this very susceptible animal there is no destruction of toxin outside the nerve centres and that the whole of the poison introduced makes its way without hindrance as far as these organs. Ehrlich, in his report to the International Congress of Medicine in Paris (August, 1900), accepted these results, as seen from his tenth
[412] and eleventh propositions: "The receptors exist, sometimes in certain tissues only, sometimes in the majority of the organs (action of tetanus poison in the guinea-pig and in the rabbit)," "...the presence of numerous receptors in the organs of less vital importance may bring about—thanks to a kind of diversion of the toxin molecules—a diminution in the susceptibility of the animal to this toxin[1]." We must here recall the differences between the susceptibility of the guinea-pig and that of the rabbit to small doses of tetanus toxin frequently repeated as in Knorr's experiments already referred to. The guinea-pig, subjected to these injections, dies in a tetanic condition long before it has received the minimal lethal dose for this species when injected in a single dose. The rabbit, on the other hand, is very tolerant of repeated doses and even rapidly acquires an immunity against five minimal lethal doses for the rabbit (injected at once). Knorr explained this difference as due to the hypersusceptibility of the nerve centres in the guinea-pig and to their acquired insusceptibility in the rabbit. The experiments of Roux and Borrel on the cerebral tetanus of rabbits vaccinated against tetanus, have demonstrated that this insusceptibility is not produced in these animals. We must, therefore, seek some other explanation. In rabbits subjected to small repeated doses, the poison is more and more prevented by certain living elements from reaching the nerve centres. Further, it is neutralised by the antitoxin which is rapidly produced. We find from Knorr's[2] researches that in rabbits antitoxin appears in the blood in cases where, affected with a transitory tetanus, their limbs remain contracted for weeks. In guinea-pigs, affected with the same form of tetanus, antitoxin in appreciable quantity is never found, even after complete recovery. All these facts accord with the hypothesis that there exist, outside the nervous system, certain living cells which absorb the tetanus toxin and produce antitoxin. Rabbits and fowls possess this property in a much

[1] *Compt. rend. Congrès internat. de Médicine de Paris*, Section de bactériologie et de parasitologie, Paris, 1891, p. 30.

[2] *München. med. Wchnschr.*, 1898, S. 321.

greater degree than do guinea-pigs. The fowl, according to Knorr, develops "a large quantity of antitoxin, whilst the tetanic symptoms are still augmenting." In this animal, as we have been able to show[1], a portion of the tetanus toxin is absorbed by the leucocytes. By [413] exciting aseptic exudations in fowls into which I had previously injected this toxin, I was able to convince myself that these exudations, much richer in leucocytes than was the blood, were also much more tetanigenic than was the blood. I observed also a more or less pronounced leucocytosis after the injection of non-lethal doses of tetanus toxin into fowls. It is possible that the leucocytes were actual agents in protecting the animal against the penetration of this poison to the susceptible nerve centres.

The great susceptibility of leucocytes to microbial toxins serves to indicate that these cells are of some importance in the struggle of the animal against these poisons. Their injection usually sets up a marked hyperleucocytosis of the blood. On this point Chatenay[2], working in my laboratory, has carried out a series of experiments on animals poisoned by bacterial (tetanus and diphtheria), phanerogamic (ricin and abrin) and animal (snake venom) toxins. He was able to demonstrate a striking analogy between them and the phenomena which occur in bacterial infections. When death takes place at the end of a very short period, the number of leucocytes markedly diminishes; if the animal lives beyond 24 hours or resists completely, a hyperleucocytosis, often of very marked character, is produced. In the guinea-pig, which is so susceptible to tetanus, the leucocytosis observed occurs even after injections of quantities of tetanus toxin equal to several lethal doses, and it is only after the introduction of an amount equal to one hundred times the lethal dose that the number of leucocytes remains stationary or shows a diminution. Here we have something analogous to what takes place against the anthrax bacillus in the same animal. The penetration of this deadly organism sets up a marked leucocytosis, but the accumulated leucocytes are incapable of seizing the bacilli or of preventing their noxious action. In other species of animals, such as the rabbit and the fowl, the intervention of the leucocytes against the anthrax bacillus, as well as against the tetanus toxin, is more effective.

If this toxin, instead of being injected in solution, be introduced along with the bodies of the micro-organisms which contain it, the

[1] *Ann. de l'Inst. Pasteur*, Paris, 1897, t. xi, p. 808.

[2] "Les réactions leucocytaires, vis-à-vis de certaines toxines," Paris, 1894.

struggle on the part of the animal takes place under more favourable conditions and even very susceptible animals may afford evidence
[414] that they offer a high resistance. Vaillard and Vincent[1] have shown that if we inject living tetanus bacilli, or the spores of these bacilli, deprived of free toxin, into guinea-pigs a great accumulation of leucocytes, which prevent the production of infection and intoxication by devouring the bacilli and their spores, takes place. The toxin contained in the ingested bacilli remains innocuous ; this affording evidence of the protective part played by the leucocytes against the toxin. The same interpretation may be offered to explain the survival of animals very susceptible to tetanus, when the tetanus poison, mixed with pounded cerebral substance or with carmine powder, is injected. In these mixtures the toxin, as mentioned above, becomes attached to certain substances of the triturated brain or to the grains of carmine. This fixation is very unstable, the toxin is readily set free ; but, when introduced into the body of the animal, the mixture induces a great accumulation of leucocytes which seize the cerebral particles and the grains of carmine and along with them take possession of the toxin. Wassermann and Takaki's experiments and those of Stoudensky are easily explained if we assume two protective acts : the first of these consists in fixing the toxin, thus preventing it from diffusing and rapidly reaching the living nerve cells ; the second is the absorption of the toxin fixed by the leucocytes,—cells endowed with receptors for the haptophore group of the toxin, but insusceptible to its toxophore group. When one of the two factors is absent, tetanus cannot be prevented. It is for this reason that in Courmont and Doyon's experiments with emulsion of the frog's brain, mixed with tetanus toxin, the inoculated animals died from tetanus in spite of an accumulation of leucocytes. This fact affords additional proof that, under these conditions, the toxin does not become anchored to the particles of the pounded cerebral substance, this anchoring being a condition necessary for the effective reaction of the leucocytes.

The absorption of the tetanus toxin becomes evident when we study in detail the phenomena produced in the experiments carried out according to Vaillard's methods with tetanus spores and those of Wassermann and Takaki with poison to which cerebral emulsion has been added, or according to Stoudensky's method with grains of carmine. When, however, it is desired to bring forward rigorous

[1] *Ann. de l'Inst. Pasteur*, Paris, 1891, t. v, p. 1.

proof of the presence of the tetanus toxin inside the leucocytes [415]
charged with spores, with granules of cerebral substance or with grains of carmine, very great difficulties are encountered. How, indeed, is it possible to demonstrate this poison fixed upon these various bodies, a poison, the presence of which cannot be demonstrated except by its injection into the animal? For this, in the study of the reaction of the organism of the animal against the poisons, it is very important to have recourse to substances, whose presence can be demonstrated more easily than can the microbial toxins. We must first have recourse to the alkaloids, especially atropin, which, in this respect, present numerous advantages. We know that rabbits resist considerable doses of sulphate of atropin, even when this poison is injected directly into the blood. On the other hand, when it is introduced into the brain, according to Roux and Borrel's method, even small quantities are quite sufficient, as demonstrated by Calmette[1], to produce a fatal poisoning. The intracerebral injection of the one-hundredth part of a dose which, when introduced into the circulation of the rabbit, produces no disturbance, in the same animal at the end of a few minutes sets up an enormous pupillary dilatation with symptoms of very lively excitation, increase of the reflexes, and general anaesthesia. These phenomena are succeeded by paralysis and death, which supervenes three or four hours after the injection. The natural immunity of the rabbit against atropin falls therefore into the same category as that against morphin. It is not due to the innate insusceptibility of the nerve cells, but to something which prevents the alkaloid from reaching these living elements. With the object of ascertaining the mechanism of this immunity, Calmette injected into the veins of rabbits a fairly large quantity of sulphate of atropin (0·2), he then bled these animals and collected from their blood the plasma and the white corpuscles, separating them by centrifugalisation. When injected into the brain of other rabbits, these constituents of the blood did not act in the same way. Whilst large doses of plasma set up merely a short period of excitation and a very transitory pupillary dilatation, corresponding quantities of leucocytes caused grave disturbances, sometimes followed by death in from seven to twelve hours. Calmette concludes from his researches that the atropin does not remain in the fluid part of the blood, since mere traces of it are found in the serum, but that it is

[1] "Cinquantenaire de la Société de Biologie," Volume jubilaire, Paris, 1899, p. 202.

[416] seized and absorbed almost immediately by the leucocytes[1]. This result has been confirmed by Lombard[2] by another series of experiments. After injecting very large quantities of sulphate of atropin into rabbits and guinea-pigs, he bled these animals and separated out the elements of their blood. Instead of introducing these elements into the brain of rabbits, he injected them into cats, animals very sensitive to atropin. The cats which received the red corpuscles and the plasma exhibited very insignificant symptoms of poisoning. Those, on the other hand, which were injected with a corresponding quantity of leucocytes, had much graver symptoms of intoxication, such as photophobia with maximal pupillary dilatation, dysphagia and persistent diarrhoea.

It is, therefore, to the absorption of the atropin by the leucocytes that naturally refractory animals owe their immunity, an immunity which is very marked in spite of the susceptibility of the nervous elements of these animals. We have been able to obtain this result thanks to the delicate physiological reactions obtained with certain alkaloids. As regards arsenic the demonstration could be pushed even further, for the absorption of this mineral poison by the leucocytes has been established by chemical analysis.

When engaged in my researches on the leucocytic phenomena in intoxications I succeeded[3] in showing that in rabbits subjected to rapidly fatal doses of arsenious acid, there is a marked diminution in the number of white corpuscles in the blood. On the other hand, in rabbits habituated to arsenic, the same doses which brought about hypoleucocytosis and death of the control rabbits, induced a considerable rise in the number of leucocytes. Later, Besredka[4] made continuous and detailed researches upon this subject and obtained most interesting results. In order to simplify the conditions of experiment, he studied the reaction of the organism of the animal
[417] after the introduction of a red trisulphide of arsenic[5], a not very

[1] The rapid disappearance of poisons from the blood is proved also by the experiments of von Behring, Dönitz, Decroly and Rousse (*Arch. internat. de Pharmacodyn.*, Gand et Paris, 1899, t. VI, p. 211) on snake venom and diphtheria and tetanus toxins, as likewise by those of Heymans and Masoin (*Ibid.*, 1901, t. VIII, p. 1) on the malonic and pyrotartaric nitrites. These poisons, within a few minutes of their injection into the veins, are absorbed by the cell elements.

[2] "Contribution à l'étude physiologique du leucocyte," Paris, 1901, p. 39.

[3] *Ann. de l'Inst. Pasteur*, Paris, 1894, t. VIII, p. 719.

[4] *Ann. de l'Inst. Pasteur*, Paris, 1899, t. XIII, pp. 49, 209.

[5] See Besredka, *op. cit.*, p. 50, for its approximate composition and distinction rom ordinary yellow trisulphide.

soluble salt, easily recognisable by its colour and markedly toxic. When non-lethal doses of this salt were injected into the peritoneal cavity of the guinea-pig, there was, first a transitory fall in the number of the white corpuscles in the peritoneal fluid, followed by a hyperleucocytosis of the most marked character. Of the leucocytes accumulated in the exudation the macrophages almost exclusively seized the yellowish-red granules of the trisulphide of arsenic. Very shortly, the whole of the salt injected was found within the peritoneal leucocytes, and the animals in which this marked phagocytosis occurred remained in good health. The ingested granules could be observed for several days in the macrophages; but in course of time, these arsenical particles were broken up into very small granules and ultimately disappeared. Here, then, we have an intraphagocytic solution of the trisulphide of arsenic and very probably a transformation of this salt into some other arsenical combination, innocuous to the animal. This soluble substance escapes from the macrophages and is finally excreted by the urinary passages.

Since the phagocytes ingest the trisulphide of arsenic and render it innocuous, it was to be anticipated that the elimination of these protective cells would lead to a fatal poisoning by doses which, under normal conditions, are readily withstood by guinea-pigs. When Besredka used sacs of reed-pith containing non-fatal quantities of the red trisulphide and introduced them into the peritoneal cavity of guinea-pigs these animals were not long in exhibiting symptoms of poisoning and died at the end of a longer or shorter period, this varying with the amount of poison introduced. Even when the phagocytic reaction had been impaired as the result of a previous injection of carmine powder, the guinea-pigs died after doses of trisulphide of arsenic which, under ordinary conditions, did not kill them. The phagocytes in this experiment devoured numerous grains of carmine and were rendered incapable of ingesting enough of the trisulphide of arsenic to save the animal. On the other hand, when Besredka set up a previous accumulation of macrophages in the peritoneal cavity of his guinea-pigs, he succeeded in rendering these animals resistant to doses of trisulphide of arsenic that, under normal conditions, were fatal. The whole of these facts converge to show that the phagocytes, thanks to their power of seizing the trisulphide of arsenic and of modifying it within them, exercise a beneficent and immunising action on the organism of the animal. [418] The analogy of the main facts concerning this protective influence

with that observed in the immunity against infective micro-organisms is indeed very considerable.

Having determined the part played by the macrophages in the resistance of the organism of the animal against a not very soluble salt of arsenic, Besredka proceeded to study the leucocytic phenomena in poisoning by soluble arsenical compounds. In his experiments he made use of potassium arsenite and he found that when lethal doses were injected the guinea-pigs showed a diminution of leucocytes in the blood in less than 24 hours, whilst with non-lethal doses, he produced a marked hyperleucocytosis. When he injected lethal doses into rabbits accustomed to arsenic, these animals manifested an increase of white corpuscles, just as in animals injected with non-lethal doses. These oscillations in the number of leucocytes, like those which have been observed after poisoning by trisulphide of arsenic, certainly indicate that the organism and its defensive cells behave in the same way to both slightly soluble and very soluble salts of arsenic. In the first case it was easy to demonstrate that the accumulation of leucocytes in the blood and in the peritoneal exudation terminated in the ingestion of the granules of trisulphide. With potassium arsenite, it was not so easy to prove the point; a chemical analysis of the elements of the blood, however, has given a decisive answer. After injecting the lethal dose of this soluble salt into rabbits accustomed to arsenic, Besredka bled them in order to separate the plasma, leucocytes and red corpuscles. Several experiments made on these rabbits gave a concordant result which this observer sums up thus: "Although the bulk of plasma and of red corpuscles was much greater than that of the leucocytes, it was in the latter only that arsenic was found" by chemical analysis. It was only in those cases where the animals survived, and manifested hyperleucocytosis, that Besredka succeeded in demonstrating the presence of arsenic in the white corpuscles.

These experiments, excluding any doubt as to the protective part played by the leucocytes against arsenical intoxication, of course suggested the idea of investigating whether the nerve elements, submitted to the direct influence of potassium arsenite, exhibit any real susceptibility to this poison. The injection of solutions of this salt
[419] into the brain demonstrated that the one-hundredth part of an ordinary lethal subcutaneous dose was sufficient to cause fatal poisoning. This fact, then, falls into line with other facts, already numerous, as to the susceptibility of the nerve centres to microbial toxins, alkaloids

and other poisons. But in the case of potassium arsenite, it was even more easily demonstrated than in the other cases that immunity natural or acquired, is connected with the absorption of the poison by the leucocytes. These cells, themselves much less susceptible to the toxic action than are the nerve elements, protect them from contact with the poison.

It is manifest that arsenic is not the only mineral substance capable of being absorbed by the phagocytes, and there are already on record well established facts in support of this thesis. Some time previous to the researches on arsenical poisoning just summarised, Kobert, then in Dorpat, set his pupils, Stender, Samoïloff, Lipsky and others[1] to make systematic researches on the fate of iron in the animal organism. For this purpose these observers made use of a very soluble preparation of iron—or better expressed, as soluble as possible—Dr Hornemann's *ferrum oxydatum saccharatum solubile*, which does not precipitate in alkaline media. They proved that a small quantity of the iron introduced into the animal is eliminated by the kidneys and the wall of the intestine, but that the greater part of the metal is arrested in the organs, especially the liver, spleen and bone marrow. The iron is there absorbed by the leucocytes which hold it for some time and then throw it into the intestine.

I have had the opportunity of observing this circulation of Dr Hornemann's soluble salt in the organism of several species of vertebrates. Some time after its introduction into the organism by the blood vessels, peritoneally or subcutaneously, the iron may be found (by means of the microchemical reaction with potassium ferrocyanide) accumulated in the various phagocytes, especially the leucocytes, the stellate Kupffer's cells of the liver and the macrophages of the splenic pulp. The non-phagocytic cells, as, for example, Ehrlich's basophile leucocytes, so abundant in the lymph of rats, take up very little of this iron, although the macrophages and microphages are full of it[2]. Against these facts Weigert[3] has advanced [420] the objection that the leucocytes absorb only the iron precipitated in the form of granules, but my own researches allow of no doubt that not only granular but dissolved iron is absorbed. This dis-

[1] *Arb. d. pharmak. Instit. z. Dorpat*, 1893, 1894, Bde VII—X.

[2] *Ann. de l'Inst. Pasteur*, Paris, 1894, t. VIII, p. 719.

[3] Lubarsch u. Ostertag's *Ergebnisse d. allg. Path.*, Jahrg. IV for 1897, Wiesbaden, 1899, S. 107.

cussion, however, loses much of its importance in view of the results obtained with potassium arsenite.

According to Samoïloff[1], soluble salts of silver in the animal organism undergo a fate similar to that of Hornemann's soluble iron salt and are absorbed by the phagocytic elements. It must be noted, further, that according to the experiments of Arnozan and Montel[2], the leucocytes absorb such drugs as calomel and salicylate of soda.

These observations all clearly show that the phagocytes must not be looked upon as cells capable of seizing merely the dead bodies of micro-organisms and of animal cells, always fearing and avoiding poisons and only able to come forward when protected by some other antitoxic function. The phagocytes no doubt often exhibit a negative susceptibility for many poisons, when these are introduced into the animal organism in too large a quantity. But these cells are most resistant to toxic substances and protect the higher elements from the poison. Under these conditions, it is quite natural to assign to the phagocytes the rôle of the fighting agents of the animal organism against poisons and we may even enquire whether these elements do not produce the antitoxins. It has been pointed out that it is very difficult to attribute this function to the cells susceptible to the toxic action,—the spermatozoa in the production of antispermotoxin, the red blood corpuscles in the development of antihaemotoxin, or the nerve cells in the production of tetanus antitoxin. Moreover since, according to Ehrlich's theory, it is only the haptophore group which excites the formation of antitoxins on the part of the elements which possess the corresponding receptors, it is quite possible that the phagocytes, thanks to the facility with which they absorb the poisons, occupy an important place as producers of antitoxins. I have already
[421] formulated this hypothesis, and several investigators, amongst whom may be cited Gautier[3] and Courmont[4], have received it favourably, though in the imperfect state of our knowledge, it cannot, as yet, be fully demonstrated. It might perhaps be objected against this hypothesis that in many instances, after the injection of micro-organisms living or dead, in spite of a vigorous leucocytic reaction the organism of the animal does not produce any antitoxin. In such

[1] *Arb. d. pharmak. Instit. z. Dorpat*, 1893, Bd. IX, S. 27.

[2] Communication to the XIIIth Intern. Congress of Medicine in Paris, 1900.

[3] "Les toxines microbiennes et animales," Paris, 1896.

[4] In Bouchard's *Traité de Pathologie générale*, Paris, 1900, t. III, 2me partie, article "Inflammation."

cases, there is clearly a development of antibodies, such as the fixatives, whose phagocytic origin may reasonably be claimed, but no true antitoxins. It must not be forgotten, however, that the various kinds of phagocytes present, amongst themselves, great differences, and that perhaps certain only of these elements are capable of producing antitoxins. When micro-organisms, living or dead, are introduced into an animal it is found that antitoxins do not as a rule appear in the fluids; in these cases the reaction is set up mainly by the microphages. The macrophages represent the principal source of antitoxins. In cases where these phagocytes ingest the micro-organism the blood exhibits an undoubted antitoxic power. Such is the case with bubonic plague in the human subject, where the micro-organism is readily ingested by the macrophages. Here we obtain antitoxic serums even after the introduction of living or dead organisms into the animal, a fact observed by Roux and his collaborators. Another fact in favour of the hypothesis I am defending is furnished to us by the cayman. As noted above, this reptile, of all known animals, supplies antitoxins most quickly and easily. In the cayman the leucocytic system is composed of eosinophile microphages filled with granules, and of macrophages. As the eosinophile cells are only very weakly phagocytic, it is the macrophages almost exclusively which intervene in the reaction against the micro-organisms. It is probable, then, that in the cayman and in animals inoculated with the plague bacillus the exclusion of the microphages from the struggle constitutes a factor favourable to the production of antitoxins and at the same time favourable to the manifestation of the activity of the macrophages.

If these latter phagocytes play the primary rôle in the excretion of antitoxins in the fluids of the body we should expect to find this [422] function exercised not only by the motile macrophages of the blood and lymph, but also by the fixed macrophages, so widely diffused through almost all the organs.

I advance this hypothesis for what it is worth, simply as a guiding idea for new researches in this field, of which so much is still unknown[1]. The brief account of the actual state of the question of

[1] Römer's recent researches (*Arch. f. Ophth.*, Leipzig, 1901, Bd. LII, S. 72) on anti-abrin accord very well with our hypothesis. He was able to demonstrate that the spleen, the bone-marrow, and the conjunctiva of the eye, when submitted to the influence of abrin, contain a notable quantity of anti-abrin. Now these three organs are very rich in phagocytes.

artificial immunity against toxins, has indicated to us that this is a problem far more difficult of solution than is that of acquired immunity against micro-organisms. The mere fact that these latter can still be found some hours or even days after their entry into the refractory animal, affords a great advantage in these researches as compared with those on toxins which are lost, often almost immediately, after their injection. Consequently our knowledge of antimicrobial immunity is more advanced than is that on immunity against the soluble products of micro-organisms.

The facts narrated in this chapter support the thesis I have defended on the subject of immunity against micro-organisms—that antimicrobial immunity in no way depends on a previous resistance against the toxins. As a general rule the immunity against micro-organisms is developed more readily than the immunity against their toxic products and at an earlier stage.

Although much still remains to be done in the elucidation of the mechanism of antitoxic immunity, the principal data acquired on the subject of this immunity have undoubtedly led to applications of the highest importance, as will be set forth in one of the following chapters.

CHAPTER XIII

IMMUNITY OF THE SKIN AND MUCOUS MEMBRANES

Protective function of the skin.—Exfoliation of the epidermis as a means of ridding the animal of micro-organisms.—Localisation and arrest of micro-organisms in the dermis.—Intervention of phagocytes in the defence of the skin.

Elimination of micro-organisms by the conjunctiva.—Microbicidal function of the tears.—Absorption of toxins by the conjunctiva.—Protection of the cornea.—Elimination of micro-organisms by the nasal mucosa.—Protection by the respiratory channels.—Dust cells.—Absorption of poisons by the respiratory channels.

Alleged microbicidal property of the saliva.—Part played by microbial products in the protection of the buccal cavity.—Antitoxic function of the saliva.

Antiseptic action of the gastric juice.—Antitoxic function of pepsin.

Protective function of the alimentary canal.—Absence of microbicidal power from the intestinal ferments.—Protective function of the bile.—Antitoxic rôle of the digestive ferments.—Favouring and retarding functions of the intestinal micro-organisms.—Destruction of toxins by these micro-organisms.

Defensive rôle of the liver. Protective function of the lymphoid organs of the alimentary canal.

Protective function of the mucous membrane of the genital organs.—Autopurification of the vagina.

In the preceding chapters the phenomena of immunity which are exhibited within the animal body in which the portals were open for the penetration of the micro-organisms and their poisons have been studied. We had to deal almost exclusively with experimental immunity, the study of which constitutes the basis of our present knowledge concerning the general problem of immunity. In natural immunity, however, things do not follow the same course. The micro-organisms and their toxins are not introduced directly into the tissues and blood by means of a syringe or other instrument. The micro-organisms have to make their own way through the skin and the mucosae, tissues which offer a resistance more or less serious and

effective ; or they may have to take up their abode in the cavities of the animal organism, in order that they may be able to inundate it with their poisons. We must here review briefly these natural barriers to microbial invasion.

The skin constitutes a protective covering of great importance in
[424] connection with the preservation against microbial invasion of the delicate parts of an animal. In many of the lower and higher animals, and even in man himself, the skin becomes the seat of a microbial flora, often very abundant, in which may be found, in addition to certain inoffensive organisms, other minute parasites more or less harmful. The pyogenic cocci, staphylococci and streptococci, are constantly found on the human skin, most frequently hidden in the depths of the canals of the hair follicles. These micro-organisms seize every favourable opportunity to attack the organism, producing such local lesions of the skin as acne, pimples, boils, and erysipelas, or even becoming generalised in the blood and tissues, as in the septicaemias and pyaemias. To the skin, therefore, must be assigned a very important function in the prevention of the invasion of micro-organisms which are found constantly on the surface of the body or which, along with all kinds of dirt, are brought there accidentally.

The skin is able to fulfil this protective function from the fact that, in most animals, it is covered with a not very permeable layer of some considerable thickness. In the majority of the Invertebrata, of all classes, the surface of the body is clothed with a chitinous layer, sometimes very thin and capable of folding and following all the movements of the body ; or again it may be impregnated with calcareous salts and very hard, as in the case of the integument of Insects and Crustacea, and the shell of the Mollusca. In all cases this cutaneous sheath constitutes a formidable obstacle to the entry of micro-organisms. Even in animals of very small size the thin cuticle is effective in preventing any invasion by these parasites. Thus the *Saprolegniae*, fungi so fatal to many aquatic animals, are often quite unable to pass through this cuticular layer. In order to pass this obstacle their germs must take advantage of some fissure or wound, produced by other causes. *Daphniae*, too, may often be observed to succeed in ridding themselves of the *Monospora* with its needle-like spores by means of a mechanism which we have already described in chapter VI. The white corpuscles of the blood surround the spores of this parasite and transform them into an

innocuous detritus. Sometimes, however, a number of these fine spores manage to perforate the cutaneous investment of the small crustacean; quite a small opening is made in the chitinous wall, which in itself is a source of no danger. As soon, however, as a spore of the *Saprolegnia* approaches this opening, it immediately begins to thrust a process through the small lesion, and from that moment the fate of the *Daphnia* is sealed. Incapable of opposing the slightest [425] phagocytic resistance to the filaments of the fungus, it is invaded throughout by the mycelium and soon dies.

The integrity of the skin being so important for the preservation of life, a fairly perfect mechanism has been elaborated for the maintenance of this integrity. All animals, no matter what their position in the animal scale, are liable to lesions and wounds of the surface of their bodies. In the *Daphniae* I have often[1] observed wounds produced by the bites of other aquatic animals. The surface of these wounds soon becomes covered with a rich microbial vegetation. The leucocytes are brought up to the injured point and there produce a protective layer; but, at the same time, a rapid proliferation of the neighbouring cells of the epidermis takes place; this closes the wound and separates the skin, so reconstituted, from the micro-organisms. Everything resumes its original order and the leucocytes soon disperse, regaining the blood stream.

These phenomena, which can be readily observed under the microscope in such small and transparent animals as the *Daphniae*, may serve as the prototype of those of a number of analogous processes in the animal kingdom. The thicker and more solid the cuticular investment, the more fully it guarantees the animal against the penetration of micro-organisms. Cuénot[2] made the observation that Crustacea, furnished with such a hard envelope as is the carapace of the Decapods, are completely defenceless from the moment parasitic micro-organisms make their way into their bodies. These intruders quietly instal themselves in the tissues, without causing the slightest phagocytic reaction, and thus bring about the inevitable death of the host. The protection of the animal in this case is, so to speak, associated with the resistance offered by the carapace.

Again, in many of the Vertebrata, the skin has a hard, thick sheath, e.g., the scales of fishes and of reptiles. Man, with his supple and not very thick skin, is less well endowed; this, however,

[1] *Virchow's Archiv*, Berlin, 1884, Bd. xcvi, S. 192.
[2] *Arch. de Biol.*, Gand et Leipzig, 1893, t. xiii, p. 245.

does not prevent him from defending himself against the entry of micro-organisms by the cutaneous path. Sabouraud[1], a well-known dermatologist, has given a very concise and at the same time very complete sketch of the part played by the skin in the protection
[426] of the body against micro-organisms ; from this author the following data are borrowed.

The epidermic layer sets up a defence by the production and expulsion of corneal cells. In the normal course of the life of the epidermis, the cells of the deeper layers, coming to the surface, become exfoliated and are thrown off. "There is thus produced, a continual exfoliation of the dead layers, and a continual eviction of such micro-organisms as are living on them. The epidermis is dense and its cells have a hard envelope ; the micro-organism is not endowed with motion, or at least not with sufficient to be of service in effecting an entrance. It can only penetrate the epidermis by multiplication *in situ*, a micro-organism originates alongside another, another in front of it, and in front of this again others. In this way they burrow between the apposed cells just as a root penetrates into the ground ; so great is the resistance of the horny cells that we never find any micro-organisms within them, but between them only" (p. 734). The epidermic cells, containing micro-organisms, exfoliate, and the skin is thus ridded of them. Frequently the process, as it goes on constantly and slowly, is invisible ; but often, on the other hand, it becomes exaggerated and manifests itself in the form of a cuticular desquamation which leads to the elimination of a large number of micro-organisms. The patient may retain "such pellicles for ten years, and even longer, without presenting anything else but these, and there are many other chronic squamous infections in which the course is uncomplicated by even an erosion or the slightest wound."

The connective tissue of the human skin is also fully able to defend itself ; it is extremely vigorous and represents a real obstructing and resisting tissue. The penetration of parasites sets up in it a thickening of the fibrous tissue ; this effects a localisation of the microbial focus. To appreciate the effectiveness of this dermic defence, we have only to compare the slow growth of lupus, a form of cutaneous tuberculosis, with that of tuberculosis of the lungs and other viscera, or the slow evolution of farcy, or cutaneous glanders, with that of the visceral form of the disease.

[1] *Ann. de dermat. et de syph.*, Paris, 1900, t. x, p. 729.

If we examine more closely the process by which the dermis surrounds the intruders with a fibrous capsule, we readily recognise in it a reaction of the macrophages of the skin. In lupus these phagocytes seize the tubercle bacilli, combining to form giant cells and giving rise to an exaggerated development of the connective tissue fibres. Moreover, when the skin is menaced with a microbial invasion, not only the local macrophages but the leucocytes are mobilised. The [427] migratory white corpuscles travel through the epidermis and the connective tissue layer. In spite of the absence of a lymphatic circulation in the epidermis, the leucocytes penetrate into this layer "and, in a section through the normal epidermis, it is very rare not to find here and there some deformed and flattened leucocyte, surprised just as it was creeping between the cells of the *rete mucosa* or of the *stratum granulosum*." Immediately that the epidermis or the dermis finds itself menaced with a microbial invasion, an accumulation of leucocytes of all kinds is produced at once; this may remain microscopic or it may assume proportions visible to the naked eye. Frequently the subjacent epithelium throws off epidermic scales which are filled with leucocytes; often also the leucocytic foci in the dermis become emptied, the micro-organisms being expelled along with their enemies the phagocytes.

The tissues of the skin proper defend themselves against micro-organisms as well as they are able; but so soon as the danger becomes serious there is sent to their succour a whole army of mobile phagocytes. This example of the defence made by the cutaneous investment may serve as a prototype of that of every other region of the body. Alongside a local action, there is always an intervention of mobile phagocytes; but when this action becomes insufficient, a much more abundant accumulation of leucocytes than is found in ordinary cases is immediately produced.

Like the skin, the mucous membranes are invested with an epithelial layer, which serves as a barrier to the entry of micro-organisms. But whilst the surface of the normal skin is dry or barely moistened by the secretory products of the cutaneous glands, the mucous membranes are always humid, a condition favourable to the multiplication of micro-organisms. Hence the mucous membranes which are most exposed to contact with the air and with external objects, always contain a larger or smaller number of organisms, amongst which the pathogenic species, notably staphylococci, pneumococci and streptococci, are the most common. The part played by

the animal organism in getting rid of these micro-organisms becomes more complicated than in the case of the defence made by the skin.

The first of the mucous membranes to be exposed to contamination by micro-organisms is the conjunctiva of the eye. At the moment of birth it is in contact with the vaginal mucous membrane and acquires from it some of its micro-organisms, both innocuous and pathogenic. Tears fulfil the function of averting the danger resulting
[428] from this proximity and from the presence of micro-organisms in the conjunctival sac in general. Ophthalmologists have shown that these tears transport the organisms into the nasal cavity by means of the lachrymal canal. To determine this point Bach[1] introduced a number of Kiel water bacilli along with pyogenic staphylococci into the conjunctival sac of various individuals. Seedings made with the tears showed a very rapid disappearance of the two organisms, which passed into the nose where their presence could be demonstrated by making plate cultures of the nasal mucus. Enormous numbers of the Kiel bacilli, introduced into the conjunctival sac, were all transferred to the nasal cavity, on the average, by the end of half-an-hour. The pyogenic staphylococci persisted on the surface of the conjunctiva for a longer period, but they also passed in large numbers through the lachrymal canal into the nose.

Certain observers, notably Bernheim[2], thought that the tears, in addition to their purely mechanical defensive action, were capable of destroying the micro-organisms by their microbicidal power. Bach[3] submitted this question to a minute examination and came to the conclusion that several species of bacteria, introduced *in vitro* into the tears of healthy persons or of those who were suffering from conjunctivitis or certain other ocular diseases, disappeared somewhat rapidly. Comparative experiments with tears previously heated to 58° and even to 70° C., in most cases gave the same results, that is to say, they caused a rapid disappearance of the organisms introduced. From these facts the author concluded that it is probably to the salts contained in the tears that their bactericidal action is due. Control experiments made with physiological saline solution and with various mixtures of mineral salts met with in the tears have been found by Bach to cause a like disappearance of the same species of organisms. Well water, and

[1] von Graefe's *Arch. f. Ophth.*, Leipzig, 1894, Bd. XL, S. 130.

[2] Deutschmann's *Beitr. z. Augenheilk*, Hamburg u. Leipzig, 1893, Hft. VIII.

[3] *op. cit. supra.*

even distilled water, gave the same result. In all these cases it is evident that, in the tears, there is no bactericidal cytase comparable with that found in the serums and other body fluids which may contain this phagocytic diastase. The experiments with heated tears demonstrate this clearly. On the other hand, these same experiments lead one to suppose that the diminution and even the disappearance [429] of the micro-organisms in the tears, is due to a large extent, and perhaps completely, to an agglutinative action of the salts, a fact which has been demonstrated by several observers.

In all these cases it is indisputable that the mechanical part played by the tears is the most important of the defences offered by the conjunctiva of the eye against the microbial invasion. That this defence is not always sufficient is proved by the frequency of conjunctivitis, as well as by the ease with which certain micro-organisms, inoculated into the conjunctival sac, set up a general infection. This is specially the case with the coccobacillus of human plague. When it is introduced into the conjunctival sac of susceptible animals (rat, guinea-pig, &c.), it passes thence into the nasal cavity and soon produces a generalised and fatal infection. The conjunctival membrane, even when perfectly intact, readily absorbs certain poisons. Everyone knows the rapidity with which atropin, when introduced into the conjunctival sac, causes dilatation of the pupil. But the mucous membrane may serve also as the port of entry for toxins of microbial origin. Several observers, and especially Morax and Elmassian[1], have demonstrated that the diphtheria poison placed upon an unbroken conjunctival membrane, where the epithelial layer is uninjured, sets up local lesions which progress very slowly but which terminate in the formation of actual false membranes. Nevertheless, it must be admitted that the intact epithelial layer of the conjunctiva exerts a certain defensive action against the penetration of toxins, although a very slight lesion of this layer will allow of the ready absorption of the diphtheria poison and the formation of false membranes.

The cornea likewise, so long as it is intact, exhibits a marked resistance against the penetration of micro-organisms and of toxins. When it becomes injured in any way its epithelium is repaired with great rapidity, as has been well demonstrated by Ranvier[2], who has shown that the walls of the wound close by a process of epithelial

[1] *Ann. de l'Inst. Pasteur*, Paris, 1898, t. XII, p. 210.
[2] *Arch. d'anat. microsc.*, Paris, 1898, t. II, pp. 44, 177.

"soldering" in a purely mechanical fashion, without the intervention of any preliminary proliferation of the epithelial elements. Thanks to this very rapid obliteration the micro-organisms are prevented from penetrating not only into the interior of the cornea, but into the anterior chamber of the eye.

[430] It has already been pointed out that the ocular conjunctiva gets rid of the introduced micro-organisms chiefly by removing them mechanically and sending them through the lachrymal duct into the nasal cavity. This, in turn, defends itself by making use of a similar method. In his experiments on the Kiel red bacillus, inoculated into the conjunctival sac of man, Bach demonstrated that in a very short time these micro-organisms are carried into the nasal cavity. He showed also that they do not remain long in the latter position and that their number decreases hourly.

Twenty-four hours after the introduction of these bacilli into the conjunctiva none, as a general rule, are to be found in the nasal mucus. This expulsion of the micro-organisms likewise takes place by mechanical means, aided by the movements of the vibratile cilia. It is evidently to this process that the mucous membrane owes its relative freedom from micro-organisms. Frequently, when examining the nasal mucus or when making cultures therefrom, one is astonished at the small number of micro-organisms found in the nasal cavities of persons in good health. Thomson and Hewlett[1] have certainly gone too far when they affirm that the upper regions [i.e. the Schneiderian membrane] of the nasal cavity are, in almost 80 % of cases, free from micro-organisms. But it is certain that in these regions we do find a small number only of the bacteria which exist in greater abundance in the lower (cutaneous) passages of the nose.

To explain this paucity of micro-organisms in the nasal cavity, Wurtz and Lermoyez[2] have assumed the existence of a bactericidal property in the nasal mucus. They affirm that the anthrax bacillus, after contact with this mucus for several hours, loses its virulence for the most susceptible animals, and that several other micro-organisms —the staphylococci, the streptococci, and the *Bacillus coli*—become attenuated under the same conditions. Others who have studied this question have come to a different conclusion. Thomson and Hewlett found that the nasal mucus is not bactericidal, although

[1] [*Med.-Chir. Trans.*, London, 1895, Vol. LXXVIII, p. 239]; *The Lancet*, London, 1896, Vol. I, p. 86; *Brit. Med. Journ.*, London, 1896, Vol. I, p. 137.

[2] *Compt. rend. Soc. de biol.*, Paris, 1893, p. 756.

it prevents the multiplication of micro-organisms. F. Klemperer, denies the bactericidal property of the nasal mucus. He could never demonstrate the destruction of micro-organisms by the mucus, and he also observed that bacteria do not multiply at all readily in this medium. These results confirm the hypothesis that the defensive [431] action of the nasal mucous membrane against microbial invasion is mainly effected by the mechanical elimination of the numerous micro-organisms which continually reach it. Amongst these organisms are some which are conspicuous for the ease with which they multiply in the body, taking the nasal cavity as a starting point, e.g. the micro-organisms of influenza, the plague bacillus, which, according to several observers, is very virulent when introduced by the nostrils[2], and the leprosy bacillus. This last, according to Goldschmidt[3], Sticker[4], and Jeanselme[5] often enters the human body by way of the nose.

It is certain that the olfactory apparatus deprives the inspired air of a large number of the micro-organisms which it carries. These organisms deposited on the mucous membrane are expelled with the nasal mucus. A number of the foreign organisms, carried by the air, may, however, surmount this first barrier and penetrate further into the trachea and bronchi, whence, helped by the movements of the vibratile cilia, they are usually expelled along with the mucus.

In spite of this double defence it has been recognised that very minute corpuscles and, amongst others, micro-organisms may overcome every one of these obstacles and reach the pulmonary alveoli. Here, under the name of "dust-cells" ("cellules à poussière")—"Staubzellen" of the German writers—located in the alveoli, are described certain large mononucleated elements which contain granules of foreign origin, usually deposits of soot, of a deep black. This permeability of the normal lung tissue for dust particles and pigmented corpuscles has been closely studied and clearly demonstrated by J. Arnold[6] and his pupils. Several observers have tried to determine whether micro-organisms, introduced by the respiratory

[1] *München. med. Wchnschr.*, 1896, S. 730.

[2] Batzaroff, "La pneumonie pesteuse expérimentale," *Ann. de l'Inst. Pasteur*, Paris, 1899, t. XIII, p. 385.

[3] "La Lèpre," Paris, 1894.

[4] *München. med. Wchnschr.*, 1897, S. 1063.

[5] *Presse méd.*, Paris, 1899, 8 avril.

[6] "Untersuchungen über Staubinhalation," Leipzig, 1885.

channels, behave like other bodies. Animals were made to inhale, or there were introduced into the trachea, cultures of bacteria
[432] pathogenic for the animals experimented upon. The results so obtained have been very contradictory. Morse[1], Wyssokowitch[2], and Hildebrandt[3], never succeeded in inducing anthrax by the introduction of anthrax bacilli into the lungs of normal animals. They concluded, therefore, that the uninjured pulmonary tissue was impermeable by virulent micro-organisms. H. Buchner[4] with his collaborators and pupils maintaining the opposite view, declare that rabbits that have inhaled anthrax bacilli or their spores always succumb to a fatal attack of anthrax. These contradictory results were attributed to differences in the methods employed, and an attempt was made to perfect the methods of research, especially to prevent the penetration of the anthrax bacilli by lesions of the trachea or by any channel other than that of the pulmonary tissue. Gramatschikoff[5], under Baumgarten's direction, undertook a series of experiments in order to determine whether it was possible for the anthrax bacillus to traverse the pulmonary tissue. He introduced through the trachea of rabbits and guinea-pigs an anthrax culture, afterwards washing the respiratory passages with a large quantity of broth or of physiological saline solution. Several of the animals so treated did not succumb to the inoculation, and Gramatschikoff concluded that it was impossible for the anthrax bacillus to make its way through the wall of the normal pulmonary tissue. He was satisfied that some of the injected organisms were destroyed in the lung, although he was unable to see how this bactericidal action was determined. In these experiments a large quantity of fluid was introduced after the bacilli ; this might wash away the bacilli and convey them to situations where they could exert no morbific action ; moreover the anthrax bacilli used were of doubtful virulence (the injections made to control the virulence in the subcutaneous tissue were in nearly every instance made with quantities of fluid greater than those introduced by the trachea), and Gramatschikoff's results
[433] could not be accepted as deciding the question. On the other hand,

[1] "Eingangspforten der Infectionsorganismen," Berlin, 1881.

[2] *Mitth. aus der Brehmer'schen Heilanstalt*, 1899, S. 297.

[3] "Experim. Unters. ü. d. Eindringen path. Microorganismen," Königsberg, 1888, [and in Ziegler's *Beitr. z. path. Anat.*, Jena, 1888, Bd. II, S. 411].

[4] *Arch. f. Hyg.*, München u. Leipzig, 1887, Bd. VIII, S. 145.

[5] Baumgarten's *Arb. auf d. Geb. d. path. Anat.* etc., Braunschweig, 1892, Bd. I, S. 450.

H. Buchner's inhalation experiments made with spores, and the study of the organs of animals so treated, leave no doubt as to the possibility of the invasion of an animal by the respiratory channels by the anthrax bacillus. Furthermore, the "rag-picker's disease" and the "wool-sorter's disease," or pulmonary anthrax, developed in man as a result of the inhalation of dust charged with anthrax spores, demonstrate most clearly that it is possible for the anthrax bacillus to enter the body by the respiratory channels. The pulmonary mycoses, produced by the penetration of the *Aspergillus fumigatus* in the human subject, offer confirmatory evidence.

In spite of the fact that the pulmonary tissue is not impermeable to pathogenic micro-organisms, it is none the less true that it exhibits a very marked resistance to infection by this channel. It is, however, neither the thickness of the wall, as in the case of the skin and the mucous membranes, nor the mechanical elimination with the help of the vibratile cilia or of the secretions, that constitute the means of defence in the respiratory alveoli. Here the cell elements are charged with the duty of ridding the lungs as much as possible of the micro-organisms which enter. Ribbert[1] and his Bonn pupils, Fleck[2] and Laehr[3], observed this fact long ago. They showed that the spores of *Aspergillus flavescens* and the staphylococci, injected into the veins or into the trachea, penetrate into the pulmonary alveoli, where they are soon seized by the "epithelial cells" and the leucocytes. Laehr observed that this phenomenon is produced at the end of a few hours, and that the ingested cocci within the phagocytes undergo a progressive degeneration and at last disappear. Tchistovitch[4], working in my laboratory, studied micro-organisms pathogenic for the rabbit—the anthrax bacillus, the coccobacillus of fowl cholera, and the bacillus of swine erysipelas—ingested by the "dust-cells" of the alveoli. He has added the important observation (already referred to in chapter IV) that these phagocytic elements are not epithelial cells [434] at all, but are really macrophages of lymphatic origin. They are not found in the alveoli of new-born animals, but soon appear there and instal themselves in such a manner that for long one was led to regard them as true epithelial cells of the pulmonary tissue. This tissue, invested with an extremely delicate covering, is incapable of

[1] "Der Untergang pathog. Schimmelpilze im Körper," Bonn, 1887.
[2] "Die acute Entzündung der Lunge," Bonn, 1886.
[3] "Ueb. d. Untergang des Staphylococcus," etc., Bonn, 1887.
[4] *Ann. de l'Inst. Pasteur*, Paris, 1889, t. III, p. 337.

defending itself against the invasion of micro-organisms, but the animal organism comes to its aid by sending a permanent army of macrophages which evict from the alveoli, so far as is possible, both micro-organisms and other foreign bodies. Under these conditions, we can readily understand that similar cells which fulfil the same protective function, are also found in the neighbouring bronchial glands. It has long been recognised that the macrophages of these glands are often crammed with various kinds of granules of foreign origin, which have made their way into the lungs with the inspired air.

Toxic substances can be absorbed by the mucous membrane of the respiratory channels. Roger and Bayeux[1] have shown that no lesion is required in order that diphtheria poison may invade the mucous membrane of the trachea, and so produce typical false membranes. The lung, we know, is accessible to gaseous toxic substances; moreover, its surface readily absorbs fluid poisons.

The protection of the digestive system is more complex than that of the respiratory passages; this is not remarkable, when we consider the greater complexity of the organs of digestion and the varied conditions which they present with regard to microbial invasion.

The buccal cavity, so exposed to the entry of extraneous micro-organisms along with the food and the external air, has a very rich microbial flora, in which Miller[2], the author of our most complete work on this subject, has recognised in man more than thirty species. Several representatives of this flora, e.g. the *Leptothrix* and the *Spirochaeta* are constantly present, and are very characteristic of the buccal cavity of man. With them are frequently found pneumo-
[435] cocci, staphylococci, and streptococci, whose pathogenic power is undoubted. Virulent diphtheria bacilli are also met with in a certain number of quite healthy persons. It is astonishing that, in spite of this state of things, wounds in the mouth heal very rapidly, and operations on the buccal cavity done with insufficient or no aseptic precaution do not, in the great majority of cases, set up infective complications of the slightest importance. After certain buccal operations we are often confronted with a complicated and open fissure; nevertheless the wound thus left exposed is not ordinarily the seat of any infection either local or generalised.

[1] *Compt. rend. Soc. de biol.*, Paris, 1897, p. 265.

[2] "Die Mikroorganismen der Mundhöhle," Leipzig, 2te Aufl., 1892.

It is often asked, how under these conditions does the mouth defend itself against the vast number of formidable micro-organisms. When the theory of the bactericidal power of the body fluids was dominant, and appeared to explain several important points in the general problem of immunity, the saliva was studied from this "bactericidal" point of view. Sanarelli[1], as the outcome of patient and laborious researches, came to the conclusion that the human saliva acted as an antiseptic and destroyed a large number of micro-organisms. It is true that he recognised its efficacy only when few bacteria were subjected to its action ; but even when the saliva was incapable of killing a large number of micro-organisms, it did not allow them to develop—it was a bad culture medium ; moreover, it had the power of attenuating the virulence of certain pathogenic bacteria, such as the pneumococcus, so frequently found in the mouth.

The conclusions of the Italian observer did not, however, meet with general acceptance. Miller[2] did not believe that the saliva exerted any bactericidal action, raising the objection that the absence of nutritive value in the human saliva for bacteria is explained by the fact that in his experiments Sanarelli employed filtered saliva, which consequently had been deprived of much of its nutritive substances,—epithelial débris, mucus, etc. Hugenschmidt[3], working in my laboratory, carried out a special research on the influence of the human saliva on micro-organisms, and arrived at conclusions quite at variance with those reached by Sanarelli. In spite of the variety of micro-organisms made use of, he could never satisfy himself that the saliva had any bactericidal property.

He sometimes saw, no doubt, a certain slowness of growth or [436]
even the destruction of certain of the micro-organisms sown at the commencement of the experiment, but this was very slight and rather exceptional. In most cases the micro-organisms, introduced into the saliva, grew rapidly, so that their number, in a very short time, became very considerable. Where the saliva brought about any diminution in the number of micro-organisms, this semblance of bactericidal action could be noted not only in the normal saliva, but also, as in the lachrymal secretion above described, in saliva

[1] "La saliva umana," Siena, 1891, and *Centralbl. f. Bakteriol. u. Parasitenk.*, Jena, 1891, Bd. x, S. 817.

[2] *op. cit.*

[3] *Ann. de l'Inst. Pasteur*, Paris, 1896, t. x, p. 545.

heated to 60° C. Against certain micro-organisms—the torulae and the staphylococci—the heated saliva acted more vigorously than did the unaltered saliva. It is consequently impossible to draw any parallel between the action of the saliva and that of the cytases.

Since the saliva often contains (according to certain authors even constantly) small quantities of potassium sulphocyanide, it seemed to be worth while to ascertain whether this salt is capable of destroying micro-organisms. The experiments carried out by Hugenschmidt, in order to settle this point, demonstrated that when given in doses comparable to those met with in the saliva, the potassium sulphocyanide exerts no bactericidal action.

Powerless as an antiseptic, the saliva fulfils an important function in ridding the mouth of micro-organisms in a mechanical way. The parotid secretion and that of the other salivary glands dilutes the bacteria and carries them from the pharyngeal cavity into the stomach. Hence, in diseases where the salivary secretion is much diminished, the mouth becomes the most important portal of entry for micro-organisms capable of setting up secondary infections. The saliva is further useful in diluting the alimentary detritus and preventing its stagnation and decomposition in the buccal cavity.

In addition to the direct mechanical part played by the saliva, it performs a very important indirect function. This fluid contains microbial products and diastases, and is capable of exciting in the leucocytes a positive chemiotactic activity. Hugenschmidt demonstrated the fact by introducing into animals small capillary glass tubes containing saliva. A certain time after being placed in position, these tubes became filled with considerable masses of immigrated leucocytes. The same result was obtained with guinea-pig's
[437] saliva, enclosed in capillary tubes and introduced into the peritoneal cavity of the same species. Here, again, the leucocytes assembled in the tubes and ingested the micro-organisms found in the saliva. The influence of the saliva on the afflux of the leucocytes must be regarded as an act important for the protection of the buccal cavity, and it is probably due to this attraction of leucocytes that lesions of this region heal so quickly. The leucocytes are very numerous in the glands of the mouth and the tonsils always supply large quantities of them.

We must not lose sight of the fact that the epithelial covering of the bucco-pharyngeal cavity also constitutes an important protective

factor. Just as on the surface of the skin, the corneal cells are in a permanent state of desquamation, so the cells in the mouth are being constantly renewed. This desquamation increases especially during mastication, when enormous numbers of cells are thrown off; after every meal there is a partial renewal of the surface of the lining of the buccal cavity. Being covered on their surface, and in their interstices charged with innumerable micro-organisms, the epithelial cells carry away with them all this population from the mouth.

The numerous micro-organisms which persist in the mouth, in spite of all these means for getting rid of them, must also play a certain part in the defence against infections. It is very probable that many of these saprophytes impede the multiplication of certain pathogenic bacteria; but at present it is impossible to define more exactly these phenomena of microbial competition. It is only because we have analogies in other regions of the body that we are able to defend this position.

The saliva, incapable of destroying the micro-organisms themselves, is able to act on their soluble products, as on certain other poisons. In this relation the action of the saliva on snake venom is most familiar. Wehrmann[1], who has made researches on this subject in Calmette's laboratory at Lille, has shown that the amylase (ptyalin) of human saliva, mixed with very rapidly fatal doses of venom, quite prevents its toxic action. Von Behring[2] reminds us on this point that the ancient Psylli (a race of northern Africa), at the beginning [438] of our era, employed their saliva as an antidote against snake bites.

Powerless to kill the micro-organisms, the saliva carries them off mechanically to the exterior or, more frequently, into the stomach. The acid medium of this great reservoir exerts a very marked effect on these microscopic organisms. It has long been recognised that the gastric juice prevents putrefaction and can arrest it even when it has become very advanced. From this observation an antiseptic action of this juice was inferred. Bacteriological researches, undertaken to determine the nature of this action, have demonstrated that several species of micro-organisms die very shortly after being placed in contact with the gastric juice *in vitro*. Straus and Wurz[3] found that even

[1] *Ann. de l'Inst. Pasteur*, Paris, 1898, t. XII, p. 510.

[2] "Allgemeine Therapie der Infectionskrankheiten," in Eulenburg u. Samuel's "Lehrb. d. allg. Therapie," Berlin u. Wien, 1899, Bd. III, S. 980.

[3] *Arch. de méd. expér. et d'anat. path.*, Paris, 1889, t. I, p. 370.

anthrax spores and the tubercle bacillus could be destroyed by gastric juice, after a prolonged sojourn in a sufficient quantity of this fluid. Comparative researches, made with aqueous solutions of hydrochloric acid, have demonstrated that the bactericidal action of the gastric juice depends solely on the amount of this acid that it contains, that is to say, the pepsin plays no part in the process. This juice exerts no true digestive action on the micro-organisms, but it destroys a certain number of them by its hydrochloric acid. This antiseptic action may also be inferred from a series of demonstrations on the exaggerated microbial multiplication in cases where the gastric juice has been poor in hydrochloric acid. Several observers have confirmed this bactericidal action of the gastric juice which is exerted specially against certain species capable of causing grave infective diseases. On the other hand, certain bacteria and other lower fungi are quite resistant to the antiseptic action of this fluid; they adapt themselves very readily to an existence in the stomach. Consequently there exists in this organ, even in animals such as the dog, whose gastric juice contains most hydrochloric acid, a special flora, whose most characteristic feature is the relative insensibility to the acidity of this medium. The Blastomycetes, along with the yeasts and the Torulae, constitute the most frequent representatives of this flora; alongside these may be grouped the Sarcinae and certain acidophile bacilli. Miller[1] has isolated several of these micro-organisms from the contents of the stomach, and has observed that, mixed with the
[439] food, they resist the action of the gastric juice, even that of the dog, whose hydrochloric acid content is greater than in man and many of the other mammals[2]. But these acidophile micro-organisms have no pathogenic power and consequently are not much to be feared. It is very doubtful whether even the infective bacteria which are easily killed by the gastric juice *in vitro*, are often destroyed in the stomach. The typhoid coccobacillus, which has shown itself to be so

[1] *Deutsche med. Wchnschr.*, Leipzig, 1885, no. 49.

[2] Amongst this acidophile flora one species merits particular attention. This is a spirillum, discovered by Bizzozero in the mucous membrane of the stomach of the dog. Salomon (*Centralbl. f. Bakteriol. u. Parasitenk.*, Jena, 1896, Bd. XIX, S. 433) has studied this organism, not only in the dog, but in the cat and Norway rat. Multiplying on the mucous membrane, the very mobile spirillum penetrates into the epithelial cells or is met with inside vacuoles. These latter being in communication with the external medium, the spirilla can readily penetrate by the openings. This fact has, then, nothing in common with phagocytosis, where it is the cell which ingests the micro-organisms by means of its amoeboid movements.

sensitive to the destructive action of the gastric juice of man, of the dog, and of the sheep, is, from the experiments of Straus and Wurz, quite capable of passing through the stomach without being affected. Stern[1], as the result of his own researches, as well as of those of his pupils, came to the conclusion that this micro-organism is not in the least affected by the gastric juice of a healthy man, containing the normal amount of hydrochloric acid. It was only in cases of hyper-secretion and of hyperacidity that the micro-organisms of typhoid fever were destroyed before they reached the small intestine.

The cholera vibrio also can pass through the stomach and its acid juice. After Koch's demonstration of the great susceptibility of this organism to acids *in vitro*, it was generally concluded that it must perish in the normal content of the stomach. Many cases have since been recorded in which the cholera vibrio was found, in times of cholera epidemics, in the faeces of healthy persons. In order to get into the large intestine it had to pass through the normal stomach. In experimental cholera in young suckling rabbits, a large number of vibrios were also found in the distinctly acid contents of the stomach, and they were seen to pass into the small intestine without any neutralisation of the acidity of the stomach taking place. This example proves, once again, that the phenomena that occur within the living body cannot be identified with those that go on in the test-tube, *in vitro*.

Whilst the acidity of the gastric juice exerts a certain influence on [440]
micro-organisms, the pepsin which it contains acts unfavourably on their toxins. There are many poisons which are readily absorbed, without being modified, by the mucous membrane of the stomach. Even the venom of snakes can, under certain conditions, produce its toxic effect as it is absorbed through the stomach. Thus, according to the experiments of Wehrmann[2], pepsin exerts a very feeble action on this poison. On the other hand, this diastase has a marked action on certain bacterial toxins. Gamaleia[3] pointed out that pepsin destroys the diphtheria toxin. Charrin and Lefèvre[4] have shown that it also weakens other microbial toxins. According to Nencki and Mmes Sieber and Schoumow-Simanowski[5], the gastric

[1] Von Volkmann's *Samml. klin. Vortr.*, Leipzig, 1898, no. 38, S. 290.

[2] *Ann. de l'Inst. Pasteur*, Paris, 1898, t. XII, p. 510.

[3] *Compt. rend. Soc. de biol.*, Paris, 1892, p. 153.

[4] *Compt. rend. Soc. de biol.*, Paris, 1897, p. 830, and Charrin, "Les défenses naturelles de l'organisme," Paris, 1898, p. 128.

[5] *Centralbl. f. Bakteriol. u. Parasitenk.*, Jena, 1898, Bd. XXIII, SS. 840, 880.

juice of the dog destroys relatively small quantities of the diphtheria poison. A gramme of the juice is capable of rendering innocuous 50 lethal doses of this toxin, but, in order that this action may be produced, a prolonged contact of the two substances is required. Since the neutralised gastric juice acts in the same way, this effect must be attributed not to the acidity of the gastric juice, but rather to the amount of pepsin it contains. This diastase acts much more powerfully on the tetanus toxin, 1 gramme of gastric juice neutralising 10,000 doses lethal for the guinea-pig. On the other hand, abrin is not modified by the gastric juice according to the researches of Répin[1], carried out in Roux's laboratory. Nevertheless, its action when administered by the stomach is feeble, and Ehrlich[2] has been enabled to vaccinate small animals against this vegetable poison by availing himself of his knowledge of this fact. Répin explains this result as due to the very slight absorption of abrin by the gastro-intestinal mucous membrane. This same factor, Répin thinks, may contribute also to the failure of various toxins when ingested. This rule, however, is not an absolute one. Thus, the toxin of the botulinic bacillus of van Ermengem[3] is not destroyed by the digestive diastases, and it is certainly absorbed by the mucous membrane of the alimentary canal. For this reason, when it is introduced by way of the stomach, it exhibits a very violent toxic activity.

The stomach, though capable, through its acid, of preventing
[441] the multiplication of certain micro-organisms, protects, very feebly, the rest of the digestive apparatus. As soon as, in the duodenum, the acidity is weakened or neutralised, the various micro-organisms commence to multiply and soon develop very abundantly.

In the animal series the intestine proper presents a very great variability, and even, in closely allied species, exhibits considerable differences. From the particular point of view which interests us these differences are very marked. Alongside insects, such as the silkworm, the larvae of cockchafers and others, whose intestinal canal contains a very rich bacterial vegetation, we have others which contain exceedingly few micro-organisms or, indeed, none at all. This last condition is represented by the caterpillars of small Lepidoptera, and notably by those of several species of clothes-moths. These differences correspond to the variety of the juices and digestive ferments met with

[1] *Ann. de l'Inst. Pasteur*, Paris, 1895, t. IX, p. 517.
[2] *Deutsche med. Wchnschr.*, Leipzig, 1891, SS. 976, 1218.
[3] *Centralbl. f. Bakteriol. u. Parasitenk.*, Jena, 1896, Bd. XIX, S. 442.

in these Invertebrata. As the physiology of digestion in these animals is as yet little understood, it is at present impossible to define clearly the conditions which regulate these phenomena. In any case, it is very probable that the soluble digestive ferments destroy the micro-organisms and prevent them from growing in the intestinal content. Otherwise it is difficult to explain why the larvae of clothes-moths, which live in old dusty textile fabrics, where the germs of bacteria are not wanting, present a digestive canal from which micro-organisms are entirely absent. The digestive juices, adapted to digest wool and even wax, are evidently capable also of digesting the bodies of micro-organisms. In other insects, which feed on vegetables and on substances less difficult to digest, micro-organisms develop in the intestinal content, as in many of the higher animals. Insects often have their intestine lined by a very delicate chitinous membrane which offers no obstacle to the absorption of the products of digestion, but prevents the micro-organisms from reaching the epithelial layer. We have here a defensive apparatus against microbial invasion, which must be the more useful because this membrane is thrown off and renewed at each moult, thus enabling the insect to rid itself at one swoop of a large number of its microscopic inhabitants.

In the Vertebrata the canal of the pancreas and that of the small intestine are always populated by a greater or smaller number of micro-organisms, amongst which bacilli predominate. We know the great difficulty experienced every time we wish to make experiments on the pancreatic digestion outside the animal body. The digestive [442] fluid, alkaline and containing many bacteria, is soon transformed into a microbial *purée*. We are then obliged to have recourse to antiseptics to arrest this development and to bring into prominence the digestive rôle played by the soluble ferments of the pancreas. This well-known fact may be used as an argument against the existence of any kind of bactericidal power in the small intestine of higher vertebrates. Even in those animals which are distinguished by the remarkable poorness of their intestinal flora, we fail to reveal the presence of bactericidal substances. The Crustacea, e.g. the crayfish, and certain worms, such as the *Ascaris*, contain few micro-organisms in their intestine. The former feed on putrescent substances, the latter inhabit the small intestine of man and animals, populated by myriads of bacteria. It might be supposed that, under these conditions, the intestinal content must contain a mass of micro-organisms or, if that be not the case, that it must contain some

substance which is powerfully bactericidal. In reality, neither one nor the other of these suppositions receives any confirmation. The intestines of the two Invertebrata I have named are very poor in micro-organisms and their contents do not exhibit the slightest bactericidal power. When a little of their contents is placed in tubes and kept at a suitable temperature it is not long before it becomes filled by a great number of bacteria of various kinds.

To explain this poverty of the microbian flora of the intestines in these examples we must postulate some kind of mechanical purification, facilitated by the peristaltic movements of the digestive canal.

Even in animals which have an abundance of micro-organisms in the small intestine, there must be produced some phenomenon which brings about the disappearance of a certain number of them. In mammals the small intestine always contains far fewer micro-organisms than does the large intestine ; in birds, the coecum is much richer in bacteria than is the rest of the digestive canal. Schütz[1] has attempted to demonstrate the disinfecting power of the small intestine in the dog by feeding it on substances to which he had added a large number of Gamaleia's vibrio (*Vibrio metchnikovi*). After convincing himself that micro-organisms perish in the digestive canal and are never found in the excrementa, Schütz introduced into his dogs
[443] a cannula, one branch of which passed into the pylorus, the other into the duodenum. By means of a small apparatus he could readily interrupt the communication between the stomach and the intestine. The vibrios, mixed with biscuit, and softened with water, introduced directly into the duodenum (whilst the stomach was kept completely isolated), penetrated into the large intestine in small numbers only. The lower part of the colon, the rectum and the excrements gave no cultures of vibrios and did not give rise to any growth except that of the *Bacillus coli*. In this case the disinfection of the intestine took place without any help from the gastric juice. Further, when Schütz killed dogs, after giving them food in which vibrios were mixed, these organisms were found in the intestine only. The gastric acidity, therefore, is not capable of killing these organisms, or of preventing them from passing into the small intestine, in which alone they were killed. It was only with the aid of purgatives, such as castor-oil or calomel, that Schütz succeeded in preserving the vibrios in the intestines and in finding them in the dejecta. This observer did not carry his investigations further and did not make out the mechanism by which

[1] *Berl. klin. Wchnschr.*, 1900, S. 553.

the small intestine destroyed such large numbers of vibrios. He supposes that alongside a mechanical factor, such as the very active peristaltic movement, there exist others, perhaps chemical processes, capable of killing these micro-organisms.

This question of the defensive action in the small intestine is, consequently, far from being settled. The data collected indicate merely that the problem is a very complex one. It has been shown, however, that very virulent bacteria may pass through the digestive canal not only without injuring the animal but even meeting their own death in this organ. The anthrax bacillus, so fatal to mice and guinea-pigs, may be swallowed by these animals without the slightest danger to them. It may then be found in the small intestine, but not in the large intestine, this proving that the gastric acidity is incapable of destroying them outright. To produce generalised anthrax by way of the intestine, it was necessary that the animals should swallow the spores of anthrax along with spiny plants, as in the experiments of Pasteur and his collaborators[1], or along with sand or powdered glass. In these cases the intestinal lesions served as the port of entry for the bacillus, the intact mucous membrane of the intestine [444] preventing their penetration. Mitchell, in an unpublished work, undertaken in my laboratory, succeeded in giving fatal anthrax to guinea-pigs, even when he fed them with spores mixed with the "crumb" of bread soaked in milk. During the whole period of the experiment the animals took no food capable of producing lesions of the wall of the intestine. But examples of infection under these conditions are altogether exceptional. In the great majority of instances the animals were not attacked. The same rule applies also to many other micro-organisms, which can be ingested with impunity although their inoculation into the blood and tissues sets up infections which are inevitably fatal. Many animals may, without running the least risk, swallow large numbers of bacteria which in man produce grave intestinal disease. Thus, it has never been possible to produce typhoid fever regularly and with certainty in any of the species of animals to which masses of typhoid coccobacilli were given by ingestion. We may recall the difficulties which so many investigators have met with in inducing intestinal cholera in laboratory animals, which are so refractory to Koch's vibrio. Only very young animals, especially unweaned rabbits, are capable of contracting fatal intestinal cholera, but these animals may contract it not only from the true

[1] *Compt. rend. Acad. d. sc.*, Paris, 1880, t. XCI, p. 86.

cholera vibrio but also from Gamaleia's vibrio. As soon as rabbits begin to feed on vegetables they acquire an immunity which is insuperable.

It is most assuredly not the digestive ferments of the intestine that protect the animal against infection through the intestine. The contents of every part of the small intestine of the Vertebrata permit an abundant development of all sorts of bacteria, and in solutions of trypsin not only do pathogenic and resistant micro-organisms grow luxuriantly, but also saprophytes and the most inoffensive bacteria. Weigert[1] influenced by this fact even saw in it an objection to the theory that the destruction of micro-organisms in the animal, notably that which is effected by the phagocytes, is to be regarded as an act of digestion. It is a remarkable fact that whilst trypsin is so powerless against micro-organisms the intracellular ferments, and especially micro-cytase, whose kinship with the group of trypsins is undeniable, are able to bring about their digestion so completely.

[445] It was thought that among the digestive fluids the bile more especially exerts a definite antiseptic power. It is undeniable that this fluid is not indifferent for certain bacteria. Talma affirms that it is bactericidal for several micro-organisms, especially the diphtheria bacillus. In many of his experiments, however, the bile proved to be incapable of killing micro-organisms introduced directly into the gall-bladder. According to the researches of Gilbert and Dominici[2] the bile does not prevent the abundant development of micro-organisms capable of setting up diseases of the biliary passages, such as the *Bacillus coli*. I have tried to prevent the multiplication of the cholera vibrio by the addition of bile, but my results were entirely negative. If the bile in an undiluted state has such a slight action upon so many kinds of bacteria, it is evident that we cannot count upon its antiseptic action when it passes into the small intestine, where it is mixed with all sorts of other substances.

The digestive fluids of the small intestine, either those that are non-bactericidal, the pancreatic juice, or those that are not very active, the bile, are, nevertheless, capable of producing a marked influence on certain poisons, and amongst others on certain microbial toxins. According to the experiments of Nencki and of Mmes Sieber and Schoumow-Simanowski (*l.c.*), trypsin is much more antitoxic

[1] *Fortschr. d. Med.*, Berlin, 1888, Bd. VI, S. 809.
[2] *Compt. rend. Soc. de biol.*, Paris, 1894, p. 38.

against the diphtheria poison than is pepsin. Thus, the pancreatic juice of both the rabbit and the guinea-pig destroys this toxin much more actively than does the gastric juice. The pancreatic juice of the dog exerts a very powerful action on the same toxin. A gramme of this fluid neutralises ten thousand lethal doses of the toxin. Wehrmann, also, found that trypsin inhibits the poisonous action of snake venom. Bile also exerts an action upon certain poisons. Mixed with diphtheria and tetanus toxins it prevents their pathogenic effect. It also neutralises the venom of snakes, as has been observed by Fraser[1], Phisalix[2] and Calmette[3]. All the venoms, when placed in contact with fresh bile for 24 hours, induce no injurious effect when the mixture is injected into normal animals. Bile, heated to 100° C., [446]
and even to 120° C., is still, though more feebly, active. To obtain these results, however, it is indispensable to prepare, beforehand, a mixture of the two fluids. When injected separately, whether at the same time as, before, or after, the venom, the bile does not prevent poisoning. The venom when injected directly into the gall-bladder of the rabbit sets up fatal intoxication to the same degree as does the same dose of venom introduced subcutaneously. Calmette, who made this experiment, explains this negative result as due to the too rapid absorption of the venom, which has not had time to be affected by the destructive action of the bile.

A protective action of the bile has been determined with regard to two viruses, the micro-organisms producing which are not, as yet, known. Koch[4] succeeded in vaccinating Bovidae with the bile of animals that had died from rinderpest, and Frantzius[5] prevented animals from contracting rabies when he inoculated into them rabic virus mixed with the bile of rabbits that had succumbed to that disease. Vallée[6] points out, however, that the bile of the normal rabbit produces exactly the same effect. Here, then, we have to do with a preventive action of the bile, as such, against the rabic virus. In the present state of our knowledge it is impossible to say whether this influence of the bile is directed against the toxin or against the unknown micro-organism. Analogy would lead us to accept the former of these two suppositions.

[1] *Brit. Med. Journ.*, London, 1897, Vol. II, p. 595.
[2] *Compt. rend. Soc. de biol.*, Paris, 1898, p. 1057.
[3] *Ann. de l'Inst. Pasteur*, Paris, 1898, t. XII, p. 345.
[4] *Deutsche med. Wchnschr.*, Leipzig, 1897, SS. 225, 241.
[5] *Centralbl. f. Bakteriol. u. Parasitenk.*, Jena, 1898, Abt. I, Bd. XXIII, S. 782.
[6] *Ann. de l'Inst. Pasteur*, Paris, 1899, t. XIII, p. 506.

The bile, active against certain poisons, does not, however, prevent poisoning by cholera toxin nor by that of botulism, two most typical intestinal intoxications.

Since diastases and the digestive juices are incapable of affecting micro-organisms and since certain of these latter perish in the intestines we must seek some other cause for their destruction. It is probable that the vital competition among the micro-organisms, whose rôle could be foreseen in the buccal cavity, is of still greater importance in relation to the phenomena of pathogenic action or of the innocuousness of infective bacteria in the intestinal canal[1]. This
[447] complex and difficult chapter, up to the present, has been studied in a very imperfect fashion. In our observations on cholera we have remarked that under certain conditions the cholera vibrios do not develop on gelatine plates, except in the neighbourhood of certain adjuvant micro-organisms such as the Torulae and the Sarcinae. Guided by this fact we have succeeded in producing intestinal cholera in suckling rabbits, with races of vibrios which, when ingested alone by these animals, remain innocuous or set up the disease only occasionally. We have convinced ourselves of the helpful action of certain representatives of the gastro-intestinal flora upon true cholera[2]. Following on these observations, it was quite natural to suppose that this flora might also contain micro-organisms capable of hindering the development and toxic action of the cholera vibrio. We have even advanced the hypothesis that these "hindering" micro-organisms in the flora of the digestive canal may explain the immunity of animals, of many human individuals, and even of the population of unattacked towns, to intestinal cholera. We should have, then, in the intestinal contents, inhabited by a number of micro-organisms and deprived of bactericidal juices, an important factor which in many cases guarantees a refractory condition. It must be stated, however, that prolonged studies, carried out with

[1] Perhaps the intestinal micro-organisms also play a part in the immunity of the animal against Entozoa. Many of the examples of this immunity are very striking. Certain intestinal worms can live only in the digestive canal of a single or of a very small number of species of animals. When we feed rabbits with a quantity of the cysticerci of the pig these pass living into the small intestine and are there transformed into true scolices. But, instead of reproducing themselves, they are expelled and never give rise to the development of taeniae. The immunity against intestinal parasites has never been made the object of special study, and it is only as a pure hypothesis that I offer this suggestion as to the part played by the micro-organisms of the intestinal flora.

[2] *Ann. de l'Inst. Pasteur*, Paris, 1894, t. VIII, p. 549.

the object of demonstrating in suckling rabbits the precise part played by these micro-organisms which prevent cholera, have not given any satisfactory results. This we attribute to our very imperfect knowledge of the microbial population of the digestive organs.

If the destruction by representatives of the normal intestinal flora of the micro-organisms which penetrate into the intestines has not as yet been satisfactorily demonstrated, the power of these latter to destroy microbial toxins cannot be doubted. We[1] have shown that a great number of micro-organisms develop well in broth cultures of the tetanus bacillus which contain a quantity of specific toxin. This toxin is destroyed under the influence of this microbial vegetation, but the production of antitoxin never results. Charrin and Mangin[2] [448] have observed similar facts.

As the destruction of bacterial toxins by micro-organisms takes place with great constancy and rapidity, it is quite natural to suppose that the same phenomenon occurs also in the intestinal canal of living animals in which pathogenic micro-organisms have succeeded in secreting their toxic products.

The liver having long been recognised as the purifying organ of the products resulting from digestion, it has been asked if it might not also play a part in the destruction of microbial poisons. Certain facts point to its inhibiting influence on the action of nicotin, atropin, and of certain other alkaloids, and we have other facts which demonstrate the power of the liver to transform into urea the ammoniacal substances arising from the activity of the digestive glands. When Nencki, Pawloff, and their collaborators[3] succeeded in making the portal vein communicate with the vena cava, and thus were able to suppress the purifying function of the liver, they found that their dogs became poisoned in consequence of the accumulation of ammonia in the animal organism.

Guided by these data as to the protective rôle played by the liver an attempt was made to apply them to the action of this organ on bacterial toxins such as the diphtheria poison. The numerous attempts undertaken in this direction have given negative results: the liver was not found to be capable of destroying this toxin. Bouchard, Charrin and Ruffer have studied the action of the liver on the pyocyanic toxin. They thought that they could make out a

[1] *Ann. de l'Inst. Pasteur*, Paris, 1897, t. XI, p. 802.
[2] *Compt. rend. Soc. de biol.*, Paris, 1897, p. 545.
[3] *Arch. de Sci. biol.*, St Pétersbourg, 1892, t. I.

certain antitoxic action of this organ, but, later, Charrin[1] convinced himself that the bacterial secretions are only "moderately modified" under these conditions, and that it is more especially the parts soluble in alcohol which undergo modification in the liver. Now, the true bacterial toxins, as is well known, are distinguished by their insolubility in alcohol. Moreover in the numerous experiments made by Roux and Vaillard and so many other observers on the tetanus and diphtheria toxins there has never been any evidence of any kind of antitoxic action of the liver.

The digestive organs are furnished throughout with a defensive
[449] apparatus against micro-organisms; this consists in an accumulation of lymphoid tissue in the form of patches or groups of solitary glands:—the tonsils, Peyer's patches, and the solitary glands of the intestine. These organs produce a large number of phagocytes which are able to come into close contact with the micro-organisms. Ribbert[2] and Bizzozero[3] have, independently or almost simultaneously, described glandular masses in the coecum of the rabbit in which they recognise the presence of many micro-organisms derived from the intestinal content. They noted that the greater number of these bacteria were within cells, and regarded this as an example of phagocytic reaction. Manfredi[4] was able to confirm this interpretation by the demonstration that the ingested micro-organisms were dead. Later, Ruffer[5] studied this question in my laboratory. He observed intestinal phagocytosis in Peyer's patches in several species of animals, and showed that the lymphoid tissue contained large macrophages filled with bacteria and microphages in process of intracellular digestion. Amongst these latter he recognised leucocytes, which in turn contained micro-organisms. The accumulation of phagocytes in the lymphoid organs of the digestive canal constitutes, so to speak, the last act of a struggle which is spread over a very wide field.

Some years ago Stöhr demonstrated[6] that the wall of the intestine, and especially the tonsils and other lymphoid organs, are traversed by an enormous number of leucocytes which execute a kind of migration towards the cavities containing micro-organisms. This con-

[1] "Les défenses naturelles de l'organisme," Paris, 1898.
[2] *Deutsche med. Wchnschr.*, Leipzig, 1885, S. 197.
[3] *Centralb. f. d. med. Wissensch.*, Berlin, Jahrg. 1885, S. 801.
[4] *Gior. internaz. d. sc. med.*, Napoli, 1886, p. 318.
[5] *Quart. Journ. Micr. Sc.*, Lond., 1890, Vol. xxx, n.s., p. 481.
[6] *Virchow's Archiv*, 1884, Bd. xcvii, S. 211.

tinual and normal condition is often termed Stöhr's phenomenon. It is evident that we have here a process of phagocytic defence in which the leucocytes, disseminated through the digestive canal, give chase to the micro-organisms that are nearest to the living portions of this organ. When we remove a particle of mucus from the surface of the tonsils of a person in good health we always find that it contains leucocytes, especially microphages, filled with micro-organisms of all kinds.

The protection of the digestive mucous membrane is a more complicated process than that of other mucous membranes, and many [450] of the points concerned therein are still obscure and need to be elucidated by further research. It might be thought that the phenomena, associated with the defence of the mucous membrane of the genital organs, being much more simple and yet of similar nature, should be much more easily made out, and that these would throw light on several aspects of the problem of the general defence of the animal. Obstetricians and gynaecologists have certainly given much attention to this question as regards the female genital organs, but we are still far from possessing a satisfactory knowledge of this subject. There already exists quite a literature on the question, dominated by the work in two volumes published by Menge and Krönig[1], but a satisfactory solution has still to be obtained.

At birth the vulva and the vagina are free from micro-organisms, but they soon become inhabited and a fairly abundant flora, in which may be recognised certain predominant species, such as the bacillus of Doederlein, is developed. Micro-organisms, therefore, can exist in the vulva and the vagina, and yet, when we introduce into these organs cultures of various bacteria, saprophytic or pathogenic, they soon disappear. We have the phenomenon to which Menge has given the name of "autopurification" of the female genital organs. He himself, as well as his predecessors, Doederlein and Stroganoff, tried to make out the mechanism of this purification. In the new-born female child the phenomenon is less complicated than in the adult. According to Menge the acidity of the vaginal secretion in these infants at first prevents the development of a large number of bacteria. Associated with this factor is a marked emigration of leucocytes, which destroy the bacteria by an act of phagocytosis, or perhaps by their products that have escaped into the vaginal mucus. As a third element to which much importance

[1] "Bakteriologie des weiblichen Genitalkanals," Leipzig, 1897.

is attributed, we must accept the intervention of acidophile bacteria which grow well in acid secretions but which hinder the development of other micro-organisms. Doederlein concludes that it is more especially to the bacillus which bears his name that the vagina owes its protection against infective germs. Menge, however, attributes this action to a whole series of bacteria.

After introducing a quantity of the *Staphylococcus pyogenes* into the vagina of new-born females, Menge found that they grew
[451] for a certain length of time. Their presence excited a great accumulation of leucocytes in the vaginal mucus, this being followed by a very marked ingestion of the micro-organisms, but it was only from the moment when the vagina became peopled with the bacteria which constitute its normal flora that the staphylococci began to disappear. This process of autopurification only ceased three days after the introduction of these bacteria. Menge asked himself whether some purely mechanical element did not contribute to rid the vagina of the micro-organisms which had entered it. To settle this point he introduced into this cavity grains of vermilion, and as these latter remained there for a longer period than did the micro-organisms, he concluded that the vagina was incapable of purifying itself by mechanical means. We must, however, in these experiments take into account the fact that the micro-organisms which Menge introduced into the vagina excited considerable reaction, accompanied by a marked leucocytosis. Under these conditions there should be produced a greater quantity of the mucous secretions which could much more readily carry off with them the micro-organisms that had come into the vagina than the smaller quantity could deal with the vermilion. It is very probable, therefore, that, just as in the case of the other mucous membranes, that of the female genital organs is capable of mechanically expelling fine particles, and especially micro-organisms.

With the object of throwing further light on the problem of the autopurification of the vagina, Cahanescu[1], working in my laboratory, undertook experiments on the females of several species of mammals. The mare, as producing the greatest amount of vaginal mucus, was selected by this observer as suitable for the settling of this question of the bactericidal power of this secretion. The result was absolutely negative, even when such an inoffensive saprophyte as the *Coccobacillus prodigiosus* was used. The autopurification of the vagina

[1] *Ann. de l'Inst. Pasteur*, Paris, 1901, t. xv, p. 842.

of the female dog, rabbit and guinea-pig, was found to be neither very marked nor very active. The micro-organisms introduced into the vagina usually remained there for some time. Of all the factors in the microbial destruction which Cahanescu was able to make out that of the accumulation of leucocytes was the most active. Sometimes he observed an extraordinary amount of phagocytosis, whilst in other experiments this was slight or even absent. Many of the leucocytes being killed in the vaginal mucus, it is possible that in some cases a certain bactericidal action of the cytases which have [452] escaped from these dead leucocytes is set up. It is true that the vaginal secretion of the mare did not exhibit this antimicrobial property *in vitro*, but in the other animals experimented upon it was found impossible to make similar experiments, the quantity of mucus being too small. In woman the acidity of the surface of the mucous membrane of the vulva and of the vagina, so frequently present, may play a certain part in the protective action against those bacteria which cannot tolerate the acid medium, but the animals studied by Cahanescu, even female dogs, do not possess this advantage, their mucous membranes usually having an alkaline reaction.

In the urinary channels this acid reaction also plays a part, as one of the defensive agencies against the penetration of bacteria. This may also be effective in man and other animals that have an acid urine. In many other animals, however, where the urine is alkaline micro-organisms do not pass into the deeper parts of the urinary organ under normal conditions. Here it is to the outflow of the urine that the bladder owes its immunity against pathogenic micro-organisms and saprophytes. When we connect two flasks containing sterilised broth in such a way that the fluid flows slowly from one of them into the other, the former never becomes contaminated by the micro-organisms which are present in the latter, in which latter the broth is soon transformed into a *purée* of bacteria, whilst in the former the broth remains unaffected and aseptic. This purely mechanical factor has been well brought out by Preobrajensky[1] as the result of work carried out in Duclaux's laboratory. The sterility of the normal urinary bladder must be attributed to a similar cause. When the urine begins to stagnate in the bladder it very readily becomes contaminated.

Since the acceptance of the view that the suprarenal capsules serve to neutralise the effect of certain toxic substances elaborated

[1] *Ann. de l'Inst. Pasteur*, Paris, 1897, t. xi, p. 699.

in the body, there has been an inclination to assume that these organs might also fulfil an antitoxic rôle against microbial poisons. The hypothesis was advanced that this function might be shared by the suprarenal capsules with the thyroid gland and with certain other problematical organs. We have already stated (Chapter v) that the suprarenal capsules, in some experiments where spermotoxin was injected into rabbits, exhibited a certain antispermotoxic
[453] power. But, up to the present, no exact fact has been observed that would favour the idea of an antitoxic action of the above-mentioned organs against bacterial toxins. Roux and Vaillard[1], in their great work on tetanus, have made experiments in this direction, but their results did not justify them in giving a positive answer to the question.

Nature does not make use of antiseptics to protect the skin and the mucous membrane. The fluids which moisten the surface of the mouth and of other mucous membranes are not microbicidal, or are so to a very slight degree, and then rather of an exceptional nature. Nature rids the mucous membranes and the skin of a large number of micro-organisms, eliminating them by epithelial desquamation, and expelling them along with fluid secretions and excretions. Nature, like the doctors of the present day who replace antisepsis of the mouth, intestine, and other organs by washing with pure physiological saline solution, has chosen this mechanical method. She avails herself of the help of inoffensive micro-organisms to prevent pathogenic micro-organisms from taking up their abode in these positions, and she is constantly sending to all the mucous membranes and the skin an army of mobile phagocytes which explore the ground and rid it of micro-organisms. When these begin to get more numerous the phagocytic reaction becomes more intense. A struggle takes place between the two living elements—phagocytes and micro-organisms. In those cases where the animal remains unaffected the former gain the upper hand.

[1] *Ann. de l'Inst. Pasteur*, Paris, 1893, t. VII, p. 65.

CHAPTER XIV

[454]

IMMUNITY ACQUIRED BY NATURAL MEANS

Immunity acquired after recovery from infective diseases.—Immunity acquired in malaria.—Humoral properties of convalescents from typhoid fever.—Preventive power of the blood of persons who have recovered from Asiatic cholera.—Antitoxic power of the blood of persons who have recovered from diphtheria.
Immunity acquired by heredity.—Absence of hereditary immunity properly so-called.—Immunity conferred by the maternal blood and by the yolk.
Immunity conferred by the milk of the mother.

It has long been known that an attack of one of many of the infective diseases brings about a refractory condition of the organism against that disease, a condition which persists for many years, and may even endure for life. Even before the microbiological era of medical science had arrived it had been fully established that a person who had recovered from small-pox might come in contact with and nurse small-pox patients without risk of contracting a second attack of the disease. The same thing has been observed purely empirically in several other infective diseases, such as whooping-cough, typhoid fever, scarlatina, mumps, etc. On the other hand it has been shown that certain infective diseases, such as fibrinous pneumonia, erysipelas, recurrent fever, and influenza, do not leave behind them the slightest trace of an immunity. It has often been observed, indeed, that after a first attack of any of these diseases there is a marked susceptibility to a second attack. Between these two extremes come the infections which are followed merely by a refractory condition of shorter duration than that which follows the diseases of the first group. The first of this intermediate group is measles, which gives rise to a relatively long immunity, then come in order bubonic plague, anthrax, cholera, etc.

It should be stated that the first attack of any of the infective diseases causes modifications more or less permanent in the organism,

and is always followed by immunity. Even in erysipelas, a disease
[455] where the relapses are so frequent that certain individuals are, so to speak, predestined to re-acquire it at short intervals, an immunity is produced, but a very transient one. Since the discovery of the streptococcus of erysipelas by Fehleisen[1], this observer, and several other investigators, have inoculated it into persons affected with malignant tumours. In the course of a series of experimental cases of treatment it was noted on several occasions that after a first inoculation, followed by typical erysipelas, a period of immunity was developed, during which the introduction of the streptococcus produced no result. It has also been observed that recurrent fever, when inoculated into monkeys, sets up a very transient but real refractory condition. In fibrinous pneumonia, also, the relapses are generally separated by periods of immunity, of longer or shorter duration.

It was generally supposed that an attack of malarial fever was not only not followed by any immunity, but that a first attack predisposed the organism to a second. Facts of this kind have often been observed and cannot now be questioned. Nevertheless, an acquired immunity against malaria is developed under certain conditions. During his travels in New Guinea, Koch[2] found that in certain regions whilst most children below ten years of age are attacked by malaria, and Laveran's parasite can be demonstrated in their blood, older children and adults are completely immune from this infection. Koch is convinced that in this instance we have an example of immunity acquired by natural means as the result of an attack of malaria at the younger age. This great observer bases his conclusion on the fact that unattacked adults, coming from districts where the children contain the parasite, do not contract malaria when they migrate to other malarial regions, whilst natives coming into these same regions from districts where malaria does not exist are soon attacked. Max Glogner[3] has attempted to explain these facts established by Koch, on the assumption that the unaffected adults simply benefit by their natural immunity and that we have here a kind of selection: the adults who are susceptible to malaria die as the result of this disease, whilst others, naturally refractory, resist and
[456] show themselves incapable of contracting the disease even in other

[1] "Die Etiologie des Erysipels," Berlin, 1883.
[2] *Deutsche med. Wchnschr.*, Leipzig, 1900, SS. 781, 801.
[3] *Virchow's Archiv*, 1900, Bd. CLXII, S. 222.

malarial regions. Glogner in support of his view cites the case of the children of the orphanage at Samarang (Java), who for many years are subject to relapses and to malarial re-infections and are incapable of acquiring the slightest immunity. According to Koch, Glogner's example cannot be compared with that of the children of New Guinea. In the former case, the natural course of the disease is interrupted by treatment with quinine, which must prevent immunity being set up; whilst, in the latter, the children are abandoned to their fate, and, receiving no treatment, slowly acquire a true immunity. It is evident that this acquired immunity in malaria is a complex phenomenon on which fresh researches must be made; but it cannot be questioned that, under certain conditions, it comes under the general rule and can be naturally acquired.

This general rule is that, in infective diseases, immunity is usually developed after a first attack. The acquired refractory condition is of very long duration in certain cases, but very transitory in others. To the discovery of the vaccination by attenuated micro-organisms, made by Pasteur and his collaborators, the objection was often made that many diseases, such as anthrax, might relapse. This is undoubtedly the case; the anthrax bacillus may attack the same individual several times; nevertheless the acquired immunity against this disease is very real, though the refractory condition lasts for one or a few years only, instead of persisting for a very much longer period, as in the case of typhoid fever, mumps, and small-pox. Bearing in mind the possibility of a relapse in the case of these infective maladies, attempts at artificial vaccination should never be relinquished.

Among the examples of immunity acquired by natural means must be cited that of syphilis, a very special case. It has long been known and demonstrated by numerous experiments on man, that individuals who have presented the primary symptoms of syphilis contract a marked immunity against a new infection. The syphilitic chancre does not relapse, and yet this very manifest and persistent immunity does not prevent the individual, immune against re-infection, from continuing to be ill and of being the field for the later syphilitic phenomena. This special refractory condition has done great service in establishing the etiology of certain diseases which we [457] were justified in suspecting to be of syphilitic origin. Many clinical observers have accepted this origin for general progressive paralysis. Others deny any causal relation between the two diseases. Krafft-

Ebing[1] has resolved this question by the application of the law of acquired syphilitic immunity. The inoculation of the syphilitic virus into ten persons attacked by general paralysis was followed by no chancre at the seat of inoculation and by no other primary or secondary symptom of syphilis. The patients with general paralysis present a real immunity against these symptoms; consequently general paralysis is a tardy manifestation of syphilis.

The acquired immunity against re-inoculation by the syphilitic virus is established immediately after the end of the period of incubation of the first infection, and is of lifelong duration[2]. Besides this very special and, so to speak, partial immunity, there exists in syphilis a second form of acquired immunity which is of a more general nature. According to the law known as the law of Baumès-Colles, the mother who suckles her infant, hereditarily infected with syphilis through the father only, enjoys a real anti-syphilitic immunity.

In tuberculosis the few facts of acquired immunity that have been observed present a certain analogy with those bearing on immunity in syphilis. A large number of well-observed facts demonstrate that a person who has suffered from scrofula or has manifest symptoms of tuberculosis properly so called, cannot count upon an immunity against pulmonary phthisis. It might, then, be supposed that no acquired refractory condition exists in connection with this disease. Koch[3] has clearly demonstrated, however, that tuberculous guinea-pigs, into which the bacilli of tuberculosis have been introduced subcutaneously, react against these bacilli in a very special manner. The presence of these micro-organisms immediately sets up an active inflammatory process at the point of inoculation; this brings about the expulsion of the bacilli with the exudation; a voluminous slough is developed, which, when shed, carries with it a large number of bacilli, a process followed neither by the formation of a permanent ulcer nor by hypertrophy of the neighbouring glands. As in syphilis, the animal has acquired immunity against re-infection by the tuber-
[458] culous virus, which, however, in no way prevents the first inoculation from becoming generalised and setting up a fatal tuberculosis of almost all the organs. Koch's observations, which have served as the basis of his researches on tuberculin, have been confirmed by other

[1] Address given at the XIIth International Congress of Medicine at Moscow, 1897.

[2] See Hudalo, *Ann. de dermat. et de syph.*, Paris, 1891, t. II, pp. 353, 470.

[3] *Deutsche med. Wchnschr.*, Leipzig, 1891, S. 101.

investigators. The reaction of the tuberculous organism against re-infection has received the name of "Koch's phenomenon."

Clinical medicine has afforded many data of the highest importance bearing on the establishment of an acquired immunity in many infective diseases; but a scientific study of the mechanism of this immunity could only be founded on the result of microbiological researches obtained during the recent period of scientific activity. The general conclusion to be drawn from these researches is that the immunity, acquired by natural means, is very analogous to that which is obtained artificially by vaccination by the various methods already mentioned. The phenomena observed in animals inoculated with the various known vaccines present a great resemblance to those that obtain during recovery from a disease contracted under natural conditions. To support this thesis it would be necessary for us to survey the mechanism of healing, which would carry us too far afield, the subject being far too vast to be summarised here. We must, then, content ourselves with a few remarks inserted for the instruction of the reader on this subject.

Those diseases against which no remedy exists are most suitable for furnishing us with important information on immunity acquired by natural means. We have already seen in the case of malaria to what point therapeutic treatment can modify the natural course of the phenomena. For this reason it will be useful to consider first the immunity acquired as the result of a first attack of typhoid fever. The immunity which develops in this example is both marked and persistent; the therapeutic intervention which might disturb the natural phenomena is *nil*.

As yet we do not know the mechanism of healing in typhoid fever. This disease affecting the human species exclusively (the experimental peritonitis of animals, set up by the typhoid coccobacillus, is distinguished by very marked differences), it is very difficult to find a means of studying it at all satisfactorily during the phase of recovery. Even in default of this knowledge, however, it is possible to gather [459] some idea as to the changes which the blood plasma undergoes, not only during the course of an attack of typhoid fever, but also during and after convalescence.

Some time ago Chantemesse and Widal[1] observed that the blood serum of persons attacked by typhoid fever acquires the property of inhibiting the experimental peritonitis set up by the typhoid cocco-

[1] *Ann. de l'Inst. Pasteur*, Paris, 1892, t. VI, p. 773.

bacillus in laboratory animals. The blood of the patient becomes "preventive." Against this conclusion the objection has been raised that in the large doses of serum employed by the above observers a protective effect can be obtained, even when using the blood of normal men, i.e. neither suffering from typhoid fever, nor having recovered from this disease. Later researches, however, have confirmed the observation made by Chantemesse and Widal. It is no doubt true that the injection of half a cubic centimetre of normal human serum into the peritoneal cavity of an untreated guinea-pig is often sufficient to render it refractory to a dose of typhoid coccobacilli fatal to the control animal. We have an ordinary protective action, such as described in Chapter x. The blood of typhoid patients is, however, capable of protecting normal animals, in doses which exhibit not the slightest protective action if normal blood be used.

The protective power of the blood serum of convalescents has been studied very carefully by Pfeiffer and Kolle[1]. In certain individuals very small quantities (0·001 c.c.) of this fluid were quite sufficient to confer on guinea-pigs an immunity against fatal typhoid peritonitis. This power was at its maximum only during the first weeks of convalescence. In one case, in which these observers were able to study the properties of the blood on two separate occasions, they found that two months after the first examination there had been a marked falling off in the acquired protective power. In a second case, where the blood was examined a year after the patient had recovered from a grave attack of typhoid fever, they found only feeble indications of this specific protective property. "Everything seems to indicate," conclude Pfeiffer and Kolle, "that the protective typhoid substances were rapidly eliminated by the blood stream. If further researches should confirm these results, as yet few in number, we might conclude therefrom that the immunity which, after an attack of typhoid fever,
[460] persists for years, frequently even for the rest of life, would be independent of the amount of ready-prepared protective substances in the blood" (*l.c.* p. 218). The facts upon which this conclusion is based confirm the general thesis that even acquired immunity is in no way the function of any humoral property.

We know that in the protective serums there is constantly found the specific fixative (the sensibilising substance of Bordet, the intermediary body or amboceptor of Ehrlich). It was, therefore, quite

[1] *Ztschr. f. Hyg.*, Leipzig, 1896, Bd. xxi, S. 213.

natural that this substance should be sought in the blood of patients who were suffering, or had recovered, from typhoid fever. Bordet and Gengou[1] easily demonstrated, by the method described in Chapter IX, the existence of typhofixative in the blood serum of two individuals convalescing from this disease.

Widal and Le Sourd[2] extended this discovery to the blood taken during the course of the disease from typhoid fever patients. The ten cases studied by them all gave a positive result, whilst all the samples of blood from persons suffering from various other diseases possessed no typhofixative. As yet we do not know whether this substance persists for any length of time after recovery or not. In this respect we have much more information concerning another humoral property of typhoid patients,—specific agglutination. Guided by the fact that, even during the course of the disease, the blood of persons suffering from typhoid fever acquires protective properties, Widal sought to find out whether the agglutinative power of the fluids of the body appears equally early. We know that his studies gave a positive answer, and that the blood of typhoid patients may have agglutinative properties from the first day of the disease. This fact was made use of by Widal to establish the serum diagnosis of typhoid fever, a method now generally used in clinical medicine. The question which most interests us at this moment is whether this acquired agglutinative property persists for any length of time after the recovery of the patient, and whether it can be employed as the measure of immunity obtained.

In certain cases the serum was found to be fairly strongly agglutinative for a considerable period after recovery had taken place. But these cases are rare, and the agglutinative power, like the protective power of the blood, usually begins to decrease very soon after recovery. Bensaude[3] observed that the former disappeared between [461]
the 10th and 95th day of apyrexia. Widal and Sicard[4] have noted in certain of their cases the complete disappearance of the agglutinative power of the blood, which took place in one case on the 18th, in another on the 24th day of defervescence. In many convalescents, fifteen to thirty days after the commencement of apyrexia, the agglutinative power begins to be attenuated.

[1] *Ann. de l'Inst. Pasteur*, Paris, 1901, t. XV, p. 289.
[2] *Bull. et mém. Soc. méd. d. hôp. de Paris*, 1901, 20 juin, p. 624.
[3] "Le phénomène de l'agglutination des microbes," Paris, 1897, p. 76.
[4] *Presse méd.*, Paris, 1896, No. 83.

Previous to these researches on the protective and agglutinative properties, Stern[1] had already put the question: May we not draw some general conclusion as to the bactericidal power of the blood serum of convalescents from typhoid fever? He found that the typhoid coccobacilli did not thrive so well in the blood serum of persons in good health as in that of convalescents, in which they give abundant cultures. Widal and Sicard (*l.c.*) subjected this question to a fresh examination, and showed that in this respect there exists no constant or marked difference. Thus, in ten samples of serums from individuals who had never been under the influence of the typhoid infection, four were found to be bactericidal for the typhoid coccobacillus. In twelve other samples, drawn from convalescents from typhoid fever, five exhibited a bactericidal power against the same micro-organism.

All the researches made on acquired immunity after recovery from typhoid fever demonstrate clearly that, in this case, it is impossible to attribute it to humoral modifications, which are usually more transitory than the immunity.

The immunity which follows an attack of cholera is far from being either as powerful or as prolonged as that which follows typhoid fever. Certain individuals have contracted cholera twice during the same epidemic, but such cases are exceptional, whilst acquired immunity, temporary at least, may be looked upon as the general rule. Many points in the pathogenesis of intestinal cholera are still obscure; nevertheless we are justified in affirming that this disease is a real intoxication by the cholera poison manufactured, in the small intestine of man, by Koch's vibrios. The action of the vibrionic toxin is sufficient to set up a grave and often fatal attack of cholera; but in the majority of cases a secondary infection by the vibrio which pene-
[462] trates into the intestinal wall, denuded of its epithelial layer, is associated with the action of the poison. Sometimes this micro-organism becomes generalised in the animal, and is found in the blood and in many of the organs.

The facts I have here briefly summarised may be utilised to explain certain characters which are found in the fluids of individuals who have recovered from an attack of cholera. Soon after the discovery of the tetanus and diphtheria antitoxins, and almost immediately after the demonstration of the protective power of the blood, taking advantage of the epidemic of Asiatic cholera, which developed in Europe from

[1] *Deutsche med. Wchnschr.*, Leipzig, 1892, S. 827.

1892, the new data began to be applied to that disease. We have already referred in a preceding chapter to the fact that the blood serum or the blood of those in good health and who have never had Asiatic cholera, is capable of preventing cholera peritonitis in the guinea-pig inoculated with Koch's vibrios. In order to obtain this protective action, the injection of a pretty large dose, about half a c.c., is necessary. This property is in no sense specific, for the same blood, injected in the same doses into guinea-pigs, will protect them not only against this vibrio, but also, and indifferently, against many other bacteria, such as the typhoid coccobacillus, the *Bacillus coli*, etc.

The blood or blood serum, coming from those who have recovered from Asiatic cholera, may, on the other hand, acquire a specific protective power. It will, indeed, prevent infections by other micro-organisms ; but, to obtain this effect, it is necessary to inject the same quantities of it as of the blood coming from normal individuals. On the other hand, when we wish to prevent cholera peritonitis in the guinea-pig, we need introduce minute doses only of the serum of persons who have recovered from an attack of cholera. Lazarus[1] was the first to make this interesting observation. In three cases of cholera studied by him, the serum withdrawn some time after recovery exhibited an extraordinary protective power : a decimilligramme of the blood serum of these patients was quite sufficient to prevent the death of a guinea-pig inoculated intraperitoneally with the cholera vibrio. Soon after, G. Klemperer[2] made a similar observation in two other cases that had recovered, but the blood, in his convalescents, was much less active than was that in the cases cited by Lazarus.

Issaeff[3], working in Koch's Institute in Berlin, examined the blood of several persons who had recovered from cholera, and found that [463] the serum had always acquired a specific protective property ; this property never developed before the third week from the commencement of the disease, and had completely disappeared as early as three months after this period. Several examples studied by A. Wassermann[4] and Sobernheim[5] fully corroborate this conclusion. Our own researches[6] on twenty-four cases indicate a very great

[1] *Berl. klin. Wchnschr.*, 1892, S. 1072 ; 1893, S. 1241.
[2] *Berl. klin. Wchnschr.*, 1892, S. 1267.
[3] *Ztschr. f. Hyg.*, Leipzig, 1894, Bd. XVI, S. 308.
[4] *Ztschr. f. Hyg.*, Leipzig, 1893, Bd. XIV, S. 42.
[5] *Hyg. Rundsch.*, Berlin, 1895, S. 145.
[6] *Ann. de l'Inst. Pasteur*, Paris, 1893, t. VII, p. 417.

variability in the protective power of the blood of persons who had recovered from cholera. We were able to demonstrate its presence in rather more than 58 per cent. of these cases. Sometimes this power was almost as marked as in the example given by Lazarus, whilst in others it was very feeble, often even *nil*. We were unable to demonstrate any relation between the gravity of the disease and the strength of the protective power of the blood. Thus, in a moderately severe case of cholera, a very small quantity of serum (0·001 c.c.) was sufficient to protect the guinea-pig from fatal cholera peritonitis, whilst in another, an extraordinarily grave case, even a quantity of 2 c.c. was incapable of producing the same effect. In these two cases the blood had been withdrawn at the corresponding period after the commencement of the disease (seventy-third and seventy-fifth days). Sobernheim (*l.c.*) found the protective power of the serum most marked in a person who had cholera vibrios in his normal dejecta, but who was always in good health and was only examined because he was living with cholera patients.

All these observations point to the fact that neither recovery from, nor immunity against, cholera can be regarded as a consequence of the protective power of the blood. This power does not manifest itself until some time after complete recovery has taken place, and then disappears too soon, that is to say at a moment when acquired immunity ought still to be maintained. On the other hand, the irregularity in the protective power of the blood indicates that this humoral property is something secondary. Since Asiatic cholera is an intoxication by the cholera toxin, we can readily understand that the protective power, resulting from the invasion of the living parts of the organism by the vibrios, should here play a part of little importance. We know already that this power is due to the presence of substances manufactured by phagocytic elements, placed in contact
[464] with vibrios. In the experimental infection of rabbits by the cholera vibrio, as demonstrated by Pfeiffer and Marx, the cells of the spleen, of the lymphatic glands, and of the bone-marrow, produce the protective substances. We have no idea of the source of these substances in Asiatic cholera in man.

Asiatic cholera, being an example of intoxication of intestinal origin, it might be supposed that the antitoxic power of the body fluids should be specially manifested after recovery has taken place. On this point our knowledge is as yet very imperfect, because it was not until after the end of the last epidemic of cholera that we learnt

how to prepare the toxin. In a case of cholera (M.S.), contracted in our laboratory, the blood serum was examined to ascertain its protective power and its antitoxic activity. This fluid, withdrawn more than three weeks after the commencement of the disease, was found to be protective only in a large dose (0·5 c.c.), in which dose even the serum of normal persons is capable of producing the same effect. It was found in an experiment with suckling rabbits that the antitoxic property of the blood serum of M.S. was *nil*. It did not prevent these rabbits from dying of intestinal cholera after the absorption of the vibrios, in spite of a dose of three c.c. of serum injected some time previously.

This experiment, unique up to the present, is, of course, insufficient to enable us to affirm that recovery from Asiatic cholera may take place without the development of antitoxic power in the body fluids. That this is so is, nevertheless, probable. In other intoxications of microbial origin, certain data have been collected which point to the same conclusion. Thus, Knorr[1] observed that the blood of guinea-pigs which had recovered from tetanus did not exhibit any antitetanic power. Vincenzi[2] made a similar observation in a man who had recovered from tetanus.

We are much better informed as to the antitoxic property of the blood of persons who have recovered from diphtheria. Klemensiewicz and Escherich[3] have studied two cases of diphtheria in which the defibrinated blood withdrawn some time after recovery was found to be protective for the guinea-pig against a lethal dose of diphtheria bacilli. This fact has been confirmed by several other observers, especially by Abel[4] and Orlowski[5], the latter of whom made his [465] researches under the direction of Escherich. In these experiments the antitoxic power of the blood was demonstrated against diphtheria toxin employed without bacilli. According to the data collected by the above authors the antitoxic property of the body fluids was not exhibited during the early days of convalescence, but was well marked in the second week after recovery. This power was maintained for a short time only, disappearing in a few months. Amongst the observations collected on this subject the most inter-

[1] *München. med. Wchnschr.*, 1898, S. 363.
[2] *Deutsche med. Wchnschr.*, Leipzig, 1898, S. 247.
[3] *Centralbl. f. Bakteriol. u. Parasitenk.*, Jena, 1893, Bd. XIII, S. 153.
[4] *Deutsche med. Wchnschr.*, Leipzig, 1894, SS. 899, 936.
[5] *Deutsche med. Wchnschr.*, Leipzig, 1895, S. 400.

esting is that made by Escherich. In an infant examined for the first time whilst it was still in good health, the blood was incapable of protecting the guinea-pig. Some time after this negative result had been obtained the child was attacked by a mild diphtheria, which gave rise to the development of antitoxin, for its blood when again examined exhibited a very high antitoxic power. This proves most clearly that even a slight attack of diphtheria is capable of producing antitoxic power in the body fluids. This observation may be utilised to explain the frequency of the presence of this property in the blood of persons in good health who, according to their own statements, have never had diphtheria. This fact has been established by the researches of A. Wassermann[1], Abel (*l.c.*), and Orlowski. According to the last observer, the blood in one-half the children in the hospital at Gratz who had not been attacked with diphtheria was antitoxic against the diphtheria toxin, sometimes even to a higher degree than was that of the children who had recovered from this disease. Wassermann has demonstrated that in adults this antidiphtheritic power of the blood is even more frequent than in children, and that it increases with age. Nevertheless, these persons affirm that they have never had an attack of the disease. To explain this very paradoxical fact, Wassermann asked himself whether the individuals whose blood was antidiphtheritic did not owe this property to the action of pseudo-diphtheria bacilli. Although incapable of causing the disease, these bacilli might, perhaps, exert a certain immunising influence and give rise to the production of an antitoxin active against true diphtheria toxin. Researches, directed to the clearing up of this point, have not led Wassermann to reaffirm his suggestion. It must be observed that the varieties of these pseudo-diphtheria bacilli are
[466] numerous, and that some of them, perhaps, may be capable of fulfilling the function suggested by Wassermann. On the other hand, it is proved that the specific and virulent diphtheria bacillus may be found in the throat of persons in good health either without inducing diphtheria, or only giving rise to a very slight form of disease of very short duration. We must bear in mind that in persons who have not had typhoid fever, but who live among patients suffering from this disease, the blood may be very agglutinative (Foerster); that in others, unattacked by cholera but containing Koch's vibrios in the intestine, the blood may acquire a high specific protective power (Sobernheim). It is probable that the same rule applies also to the

[1] *Ztschr. f. Hyg.*, Leipzig, 1895, Bd. XIX, S. 408.

case of diphtheria, and that, consequently, the blood of persons in good health, but containing the diphtheria bacillus in their bodies, may acquire antitoxic power.

This humoral power, once developed, may even be transmitted from the mother to the foetus and so become hereditary. Abel (*l.c.*) examined the blood serum of four adult women, taking it from the placenta after parturition. Each time it was found to be distinctly antitoxic against the diphtheria toxin. Later, Fischl and Wunschheim[1], working in Chiari's laboratory in Prague, studied the blood of new-born children from the same point of view. They showed that in the majority of cases this fluid prevents the production of a fatal disease in the guinea-pig, in spite of the injection of several lethal doses of very virulent diphtheria cultures. The blood of new-born children is equally capable of neutralising the diphtheria toxin, that is to say, of protecting animals against poisoning by this toxin. The above observers do not doubt that this antitoxic power comes directly from the maternal blood through the placental circulation. This fact appears to throw some light on the phenomena of immunity acquired by heredity.

Until quite recently we have had very vague notions as to the possibility of transmitting to descendants the immunity contracted as the result of recovery from an infective disease or after vaccination. It has long been known that natural immunity may be transmitted hereditarily. Certain families or certain races are characterised by a special insusceptibility to certain infective diseases. It must even [467] be admitted that this innate immunity has been transmitted from generation to generation. It is quite otherwise with acquired immunity. We know that as a rule the characters acquired during life are not transmitted to descendants; it is only in special cases, in the very lowest organisms, such as the bacteria and their allies, that we may observe the conservation of certain acquired characters through an infinity of generations. The attenuation of bacteria or the absence of the formation of spores, once acquired under special conditions, may thus be transmitted to their descendants who develop and live under normal conditions.

After the discovery of anthrax vaccine by Pasteur, Chamberland and Roux, and an attempt had been made to vaccinate large flocks of sheep, it was an easy matter to investigate whether immunity acquired by the parents was transmissible to their offspring. Several

[1] *Prag. med. Wchnschr.*, 1896.

observers, amongst whom I may specially cite Chauveau[1], Rossignol and Cienkowski, got together a certain number of data bearing on this question. These data showed distinctly that, in certain cases, the lambs born from vaccinated sheep presented, from birth, an undoubted resistance to the anthrax bacillus. This fact, however, was neither constant enough nor sufficiently marked to enable us to count upon the young animals being in a refractory condition, and thus avoid having to submit them to vaccination by the two Pasteur vaccines. This necessity threw into the background the researches on the hereditary transmission of acquired immunity. It was only much later that this question was again taken up on a purely theoretical basis. Ehrlich[2], to whom science is indebted for so many works of the highest importance upon immunity, again took the initiative in exact and minute researches upon the heredity of immunity, acquired as the result of vaccination against toxins. In this relation he studied the immunity of the descendants of animals immunised against phanerogamic toxins, such as ricin, abrin and robin, and later, in collaboration with Hübener[3], that of the offspring of animals vaccinated against tetanus toxin. Ehrlich proved very clearly that the antitoxic immunity acquired by the father is never
[468] transmitted to his progeny. This fact alone is quite sufficient to show that it is not a true immunity that is met with in young animals born of mothers who have acquired a refractory condition; true immunity is transmitted by the sexual elements, the spermatozoon and the ovum. Certain observers, Tizzoni[4] and his collaborators Cattani and Centanni, thought they could overthrow the rule established by Ehrlich. They believed that the male rabbit, vaccinated against rabies, was capable of transmitting its immunity to its progeny. Charrin and Gley[5] expressed the same opinion as regards animals of the male sex vaccinated against experimental pyocyanic disease. But the very precise experiments of Wernicke[6],

[1] *Ann. de l'Inst. Pasteur*, Paris, 1888, t. II, p. 69.

[2] *Ztschr. f. Hyg.*, Leipzig, 1892, Bd. XII, S. 183; Brieger u. Ehrlich, *Deutsche med. Wchnschr.*, 1892, S. 393.

[3] *Ztschr. f. Hyg.*, Leipzig, 1894, Bd. XVIII, S. 57.

[4] *Centralbl. f. Bakteriol. u. Parasitenk.*, Jena, 1893, Bd. XIII, S. 81; *Deutsche med. Wchnschr.*, Leipzig, 1892, S. 394.

[5] *Compt. rend. Acad. d. sc.*, Paris, 1893, t. CXVII, p. 365; *Rer. gén. d. sc. pures et appliq.*, Paris, 1896, p. 1.

[6] *Festschr. z. 100-jahr. Stiftungsf. d. med. chir. Friedr. Wilhelms-Instituts*, Berlin, 1895.

Vaillard[1] and Remlinger[2] upon a whole series of infective diseases and intoxications, such as diphtheria, cholera peritonitis, anthrax, experimental typhoid septicaemia, etc., showed conclusively the correctness of Ehrlich's results. Well-vaccinated males, even when hypervaccinated, never transmit their immunity to their descendants. This acquired property, like so many others, is not hereditary in the strict sense of the word. The females, on the other hand, with rare exceptions, transmit their acquired immunity to their young, but this transmission can in no way be attributed to the ovum ; it is here, then, no longer a question of hereditary immunity properly so called. According to Ehrlich the female furnishes in her blood plasma the antitoxin which passes into the circulation of the foetus. In all respects this is allied to the so-called passive immunity (or antitoxic immunity of von Behring). It is due entirely to the direct introduction of antitoxin, manufactured by the cells of the maternal organism, into the body of the progeny. The living elements of the foetus play no part in it, and it is for this reason that the antitoxins and immunity in the new-born animal disappear so very rapidly,—within a few weeks after birth. Wernicke accepts the views of Ehrlich in their entirety. He found that the immunity of female guinea-pigs was transmitted to the new-born animal ; but this [469] hereditary transmission was exhausted in a single generation ; it was not found in the second generation. Wernicke was able to demonstrate that the refractory condition in guinea-pigs, born of mothers vaccinated against diphtheria, persisted for three months. Vaillard found that it was retained in certain cases for an even longer period,—up to the fifth month. On one occasion he even observed the transmission of the immunity to a second generation. A female guinea-pig, born of a mother immunised against tetanus, gave birth to a young one which, when tested a month after birth with a ten times lethal dose of the toxin, contracted merely a slight tetanus.

From this fact, as well as from the fact that the immunity of the young ones born of vaccinated mothers persists longer than does that conferred by the injection of antitoxic serum, Vaillard concludes that there exists a kind of hereditary immunity which is "fixed" by the cells. He thinks that not only the antitoxins and other antibodies but also certain living elements, especially the leucocytes, are able to pass from the maternal blood into that of the foetus and to

[1] *Ann. de l'Inst. Pasteur*, Paris, 1896, t. x, p. 65.
[2] *Ann. de l'Inst. Pasteur*, Paris, 1899, t. xiii, p. 129.

transmit to it the properties acquired by the mother. At this point we may recall the facts demonstrated by von Behring and Ransom that antitoxin persists much longer in the blood of an animal when it is introduced with the serum of the same species. (We have described these observations in Chapter XII.) Now, since in hereditary transmission the antitoxin passes over with the blood plasma of the same species, whilst in the experiments on antitoxic immunity it is generally injected with the serum of a different species, it is easy to understand that the former should persist for a longer period than the latter. It is, therefore, very probable that this immunity of the offspring from vaccinated mothers is not in any way a case of true hereditary immunity, but is due simply, as maintained by Ehrlich, to the passage of ready prepared antibodies from the mother to the foetus. In the immunities against diphtheria and tetanus we have the direct passage of antitoxins; in transmitted immunity against infection by the vibrios of Koch and Gamaleia, so carefully studied by Vaillard, we have, very probably, the passage of corresponding fixatives from the mother to the foetus.

Dzierzgowsky[1] in a recent study on hereditary immunity denies
[470] the passage of antibodies and toxins through the placenta. He thinks that the foetus does not acquire its immunity through the blood of the mother, but at a very much earlier period. The ovum contained in the Graafian follicle would, according to this observer, come in contact with a fluid very rich in antitoxin, whence it might imbibe the necessary amount of this antibody to ensure the immunity of the new-born animal. Dzierzgowsky bases this opinion on experiments in which antidiphtheria serum injected into pregnant goats and dogs did not produce any antitoxic power in the blood of the foetus. But in the experiments on these animals the injections consisted of the serum of the horse—a different species. This must modify, profoundly, the conditions of the passage of the antitoxin through the placenta.

Dzierzgowsky made a single experiment upon a mare, immunised with diphtheria toxin, and its foal. Whilst the serum of the former was markedly antitoxic, that of the foal did not possess this property in the slightest degree. Hence the conclusion that the antitoxin of the mother had not passed into the blood of the foetus. But the blood of the foal was not withdrawn until some ten months after birth. Now, as the so-called hereditary immunity only lasts for a very short time

[1] *Arch. d. Sci. biol.*, St Pétersbourg, 1901, t. VIII, p. 211.

Dzierzgowsky's experiment supplies no evidence against the passage of antitoxin through the placenta.

In order to prove that the immunity against toxins may really be acquired by the ovum, Dzierzgowsky[1] carried out a series of experiments with the eggs of fowls immunised against diphtheria toxin. The yolk of the egg, in accordance with the discovery made by F. Klemperer, contained antitoxin; and this antitoxin passed into the blood of the hatched chickens. These facts, though in themselves very interesting, cannot be used to refute the view that antitoxins pass through the mammalian placenta. It is true that this view is perhaps not yet completely proved, but it accords well with all the known facts. Thus, the frequent presence of diphtheria antitoxin in the blood of new-born infants is explained much better on the assumption that it passes through the placenta than that it is due to an immunisation of the ovum surrounded, in the Graafian follicle, by antitoxic fluid. It is difficult to conceive how this immunity could be so fully retained during the nine months of pregnancy.

In support of his interpretation of the phenomenon of immunity [471]
transmitted by the mother to her progeny Ehrlich invokes his beautiful discovery of the immunity conferred by the maternal milk. A vaccinated female is capable of communicating to her young a portion of the antibodies manufactured in her organism, not only by the blood channels, but also, in certain cases, by the milk with which she feeds her young.

The transmission of antitoxins by milk has been demonstrated by Ehrlich, and this has since been confirmed by many observers (see Chapter XII). When Ehrlich found that the immunity of the progeny is retained for a longer time than is that which is conferred by injections of antitoxic serum, he conceived the idea of investigating whether the cause of more prolonged retention did not reside in the transmission of the maternal antitoxin by the milk. With the object of verifying this he took, at the moment when they had given birth to young, unvaccinated mice and mice that had been vaccinated against various toxins (ricin, abrin, tetanotoxin). He so changed the progeny that the vaccinated mothers nourished the young born of the normal mice, whilst the normal mothers suckled the offspring of the vaccinated mice. The result of these ingenious and delicate experiments fully confirmed his anticipations. The vaccinated mice transmitted their immunity not only to the young ones to which they had

[1] *Arch. d. Sci. biol.*, St Pétersbourg, 1901, t. VIII, p. 421.

given birth but also to those they had merely nourished with their milk. This observation proved, to demonstration, that the antitoxins are absorbed by the alimentary canal, a very important fact from several points of view. Later researches have shown that only very young mice are capable of assimilating antitoxin through the intestinal wall. Adult mice, fed by Ehrlich with quantities of antitoxic milk, acquired neither immunity nor any antitoxic property of the blood. Later, Vaillard (*l. c.*) was able to show that even the young of other species of animals such as the guinea-pig and the rabbit are incapable of appropriating the antitoxins from milk by the alimentary canal. He repeated Ehrlich's experiments with new-born guinea-pigs and rabbits which he caused to be suckled by mothers vaccinated against tetanus. These young rodents, so treated, were found to possess no immunity whatever; they were not able, therefore, to absorb the antitoxin
[472] found in the milk of their nurses. Remlinger (*l. c.*) made similar experiments with young guinea-pigs and rabbits suckled by foster mothers which had been vaccinated against the coccobacillus of typhoid fever. As in Vaillard's experiments, the result was negative, the milk of the foster mother did not communicate any refractory condition to the nurselings. Remlinger drew the same conclusion from his researches on the transmission of the agglutinative property of the body fluids. When female rabbits and guinea-pigs are vaccinated during gestation the young ones acquire, along with the immunity against the typhoid coccobacillus, a certain agglutinative power of the blood serum. When, however, these vaccinated females suckle the progeny of non-vaccinated mothers the agglutinative power of the milk of the foster mother never passes into the blood of the nurselings. Some years before this, Widal and Sicard[1] had demonstrated the same fact that young rabbits and new-born kittens, when fed with agglutinative milk, acquired no power of agglutinating the typhoid coccobacillus. They agreed with Ehrlich, however, that the blood serum of young mice fed with agglutinative milk acquired the power of agglutinating the typhoid micro-organism.

As it was important to determine whether the human subject was capable of acquiring a certain immunity by absorbing antibodies contained in the milk, the study of this question was taken up, especially from the point of view of agglutinative power. Although the relations of this agglutinative power with immunity are very problematical it would be interesting, bearing in mind the analogy

[1] *Compt. rend. Soc. de biol.*, Paris, 1897, p. 804.

between the agglutinative, antitoxic, and protective properties, to ascertain whether the ingestion of agglutinative milk can confer any agglutinative property on the blood serum. Numerous researches in this direction were carried out in connection with typhoid fever. Widal and Sicard (*l. c.*) caused a person to drink daily (for a period of three weeks) half a litre of milk coming from an immunised goat, a milk which powerfully agglutinated the typhoid coccobacillus. The blood, examined on several occasions, never showed the slightest agglutinative power. This experiment goes to prove that, in the adult human subject, the agglutinin does not pass from the alimentary canal into the circulation. May it not perhaps be otherwise in infants which are fed on milk only? An observation by Landouzy and Griffon[1] seemed to confirm this supposition. They first demonstrated the agglutinative power of the blood serum in a woman who had contracted typhoid fever three months after her lying-in. Being [473] a mild attack the woman continued to suckle her child during the whole course of the fever. On examination of the blood of the infant it was found that the serum agglutinated the micro-organism of typhoid fever. These observers did not measure the agglutinative power of the blood, either in the infant or in the mother. This omission deprives their observation of value since it is now recognised that normal human blood fairly frequently exhibits some power of agglutinating the typhoid coccobacillus. For diagnostic purposes it is necessary, therefore, always to measure this power in order to be sure that it is higher than that of the normal blood.

It is all the more difficult to draw any positive conclusion from the observations of Landouzy and Griffon because in several similar cases the result has been entirely different. Thus Achard and Bensaude[2] have shown that the blood of an infant, suckled by a nurse attacked by typhoid fever and whose serum became distinctly agglutinative, was incapable of bringing about clumping of the typhoid coccobacilli. Schumacher[3], working in Fraenkel's laboratory in Halle, studied a case with very great care. A woman gave birth at full term to an infant whose blood serum exhibited a certain agglutinative power. The mother suckled the infant from its birth. Although her milk manifested a very considerable agglutinative property, the blood of the child exhibited not only no increase in

[1] *Compt. rend. Soc. de biol.*, Paris, 1897, p. 950.
[2] *Semaine méd.*, Paris, 1896, p. 303.
[3] *Ztschr. f. Hyg.*, Leipzig, 1901, Bd. XXXVII, S. 323.

agglutinative power but a marked diminution. The agglutinin of the maternal blood had not passed into the fluids of the child.

From the point of view of the impossibility of acquiring immunity by suckling, therefore, the human subject may be grouped with the guinea-pig, rabbit and cat. Up to the present the mouse is the only exception. It would be very important, with the object of finding a means of communicating immunity by way of the intestine, to study the precise conditions which govern this phenomenon. In hereditary immunity, or rather in what appears to be such, those cases where the new-born animal exhibits a resisting power induced by the vaccination to which it has been subjected in the womb of the mother must be borne in mind. We have already cited the example given by Remlinger of rabbits and guinea-pigs born refractory against the typhoid coccobacillus, which had been injected into the mother
[474] animals. In those cases where the vaccination of the mothers has been carried out during the period of gestation the immunity of the young ones is more permanent than when it was completed before that period. Into this same group come those cases where women, vaccinated during the course of pregnancy, give birth to infants refractory to vaccine. Similar facts have been reported by veterinary surgeons with regard to sheep-pox ; Arloing, Cornevin, and Thomas[1] have offered similar demonstrations with regard to symptomatic anthrax.

These results may be more or less closely associated with those where the child attacked by an infective disease immunises the mother. Such facts are rare. We know that a healthy mother may give birth to a syphilitic child ; the affected father introducing the virus with the sperm, the contaminated foetus contracts the disease and the new-born infant is syphilitic. According to Ehrlich and Hubener (*l. c.* p. 54), the foetus instead of infecting the mother sets up in her a refractory condition. It must be confessed that as yet we do not understand the mechanism of this immunity ; but in any case we have here to do with an example of immunity naturally acquired under very special conditions.

Here again must be recognised another form of immunisation :— where the child born of a syphilitic mother remains healthy and contracts syphilis neither by the breast nor through the kisses of the mother. Here, undoubtedly, we have an immunity against syphilis acquired in the womb of the mother, who may, however, readily com-

[1] "Le charbon bactérien," Paris, 1883, p. 184.

municate her disease to other persons by means which are without effect on her own infant. This example comes under the law of Profetta. Here again the mechanism of the acquired immunity is absolutely unknown.

It must be admitted that, generally, we are still very imperfectly informed concerning immunity as acquired by natural paths. In cases where this immunity is developed as the result of an attack of an infective disease the phenomena observed closely resemble those that have been observed after vaccination by living, fully active, or attenuated viruses, by micro-organisms which have been killed, or by the products of these micro-organisms. These vaccinations which bring about isopathic (von Behring) or active (Ehrlich) immunity give rise to transient and mild diseases and are confined almost completely to the diseases contracted by natural means which terminate in [475] recovery and give rise to a refractory condition. The immunisation of the foetus comes into the same series.

On the other hand, the immunity which was believed to be hereditary and which results merely from the direct passage of the antibodies of the blood or of the milk of the mother to the foetus and to the child come into a group of cases characterised by what Ehrlich has termed a condition of passive immunity. We have already discussed (Chapter x) the thesis that this term "passive" is applicable only in rare cases. Most frequently it is necessary that the living cells of the organism which receives the antibodies—antitoxin, fixatives or others—should contribute their quota in order to ensure the refractory condition. This rule is undoubtedly applicable to the examples of immunity acquired by the new-born progeny of unaffected mothers.

[476]

CHAPTER XV

PROTECTIVE VACCINATIONS

Vaccinations against I. Small-pox.—II. Sheep-pox.—III. Rabies.—IV. Rinderpest.—V. Anthrax.—VI. Symptomatic Anthrax.—VII. Swine Erysipelas.—VIII. Pleuro-pneumonia in the Bovidae.—IX. Typhoid Fever.—X. Plague.—XI. Tetanus.—XII. Diphtheria.

In the preceding chapters I have attempted to present to the reader a general view of the phenomena of immunity against infective micro-organisms and against their toxic products. I shall now attempt to give a review of the facts acquired in connection with the prevention of the infective diseases of man and of the chief domestic animals by means of vaccination. Vaccinations as we know can be carried out either with viruses the constituents of which have not as yet been recognised, with micro-organisms grown on various nutrient media, with virulent or attenuated micro-organisms, or with microbial products deprived of the micro-organisms by which they have been built up. In addition to these methods we may vaccinate with protective or antitoxic serum and other body fluids, with normal serum, or with a whole series of fluids not excepting water.

I. *Vaccination against small-pox.*—We naturally commence the series with vaccination against small-pox, which is one of the oldest and one of the best known, having been practised in every country in Europe for more than 100 years. Small-pox, a very contagious and fatal malady, was very rife in the 18th century. Large cities like London and Paris were severely affected. One-tenth of the total mortality was due to this disease. According to statistical information, very exact for that epoch, the deaths from small-pox in London
[477] during the course of the second half of the century (1751—1800) numbered more than 100,000 (102,112) persons. During the first

half of the same century this disease caused great ravages in France, especially in Paris, where, according to certain statistics (Haeser), about 14,000 persons died in 1716.

Variolisation or "inoculation" coming to Europe from the East, had come into extensive use when, at the end of the 18th century, the discovery was made that cow-pox, the varioliform disease of the Bovidae, produced in persons who milked cows suffering from this eruption an immunity against small-pox. This idea, popular in origin, was known to breeders in England, France, Germany, and Holland; we have thus an indication that this knowledge must date from a fairly distant period. Jenner gave the question a scientific and experimental basis, and it was only after his intervention that vaccination by the contents of the pustules of cow-pox began to spread more generally. During the 19th century an immense amount of material bearing on this question was collected; we have thus been enabled to attain absolutely exact results, and that in spite of the very imperfect state of our knowledge on the etiology of small-pox and of cow-pox. Long ago Chauveau[1] demonstrated that the virus of these diseases must be organised, because that of the vaccine would not pass through a filter. This organism has been carefully sought, but sought in vain in spite of all improvements in micro-biological methods. It was thought that the cocci so often found in the contents of the vaccinal pustule was the specific micro-organism of cow-pox. Such was the opinion of the illustrious botanist Cohn[2]. It was soon shown, however, that this was not the case. The cocci, principally staphylococci, are "secondary" micro-organisms which may be absent from the vaccine without its losing anything of its action. A search was then made for the micro-organism of the vaccine among the protozoan organisms. L. Pfeiffer[3] announced the discovery of a species of vaccinal *Amoeba*. Guarnieri[4] has even described various stages in the reproduction of this hypothetical parasite; but Salmon[5] demonstrated, in a work carried out in the [478] Pasteur Institute, that we had here to deal merely with leucocytes which had entered epithelial cells and had there undergone marked

[1] *Compt. rend. Acad. d. sc.*, Paris, 1868, t. LXVI, pp. 289, 317, 359.

[2] *Virchow's Archiv*, 1872, Bd. LV, S. 229.

[3] *Monatssch. f. prakt. Dermat.*, Hamburg, 1887; "Die Protozoen als Krankheitserreger," Jena, 1891, S. 184.

[4] *Arch. per le sc. med.*, Torino, 1892, t. XVI, p. 403.

[5] *Ann. de l'Inst. Pasteur*, Paris, 1897, t. XI, p. 289.

degeneration. Funck[1] thought that he was able to confirm the discovery of the sporozoon of vaccinia, but his error was easily demonstrated (Podwyssozki and Mankowski)[2]. Up to the present, then, we have no knowledge of either the micro-organism of small-pox or of that of vaccinia. We still employ, as formerly, the virus taken from the vaccinal pustule. Even the relations which exist between the two viruses and the two diseases which they have set up have not yet been settled. Several authors believe that the bovine disease is only a modified and attenuated form of human small-pox; whilst others maintain that we have two very different exanthemata, one of which—cow-pox—is capable of setting up immunity not only against itself but also against small-pox.

For a long time, in order to vaccinate against small-pox, the contents of the vaccinal pustules which formed on the human subject after an original inoculation of the virus of cow-pox were employed. But a number of cases of infection by syphilitic virus and certain other accidents caused this method to be abandoned. A number of years ago, however, there spread throughout Europe and into several countries of other continents another method, which consists in vaccination by "animal lymph," that is to say, by the contents of pustules developed on the skin of the calf. This method was first carried out at Brussels in 1868, under the direction of Warlomont, at the Institute founded by the Belgian Government for the preparation of vaccine. The original virus came from a genuine case of cow-pox and has since been kept up by uninterrupted passage from calf to calf. The virus is introduced into the shaved skin of the region between the groin and the udder as far forward as the umbilicus. It is inoculated superficially into the epidermis by cuts one centimetre long. At the points of inoculation characteristic pustules develop; from these the vaccinal content is withdrawn, on the fifth day in summer or the sixth in winter. The contents are removed by pressure and by scraping the pustules. The scrapings are mixed with water and glycerine. The vaccine thus prepared is put into small glass tubes which are sealed at both ends. This method, with slight modifications, has extended to many other countries, and is
[479] carried out either in private establishments or in State institutions as in Germany. For the purpose of purifying the vaccine it is

[1] *Deutsche med. Wchnschr.*, Leipzig, 1901, S. 130; *Brit. Med. Journ.*, London, 1901, Vol. I, p. 448.

[2] *Deutsche med. Wchnschr.*, Leipzig, 1901, S. 261.

diluted and then allowed to sediment or it may be subjected to centrifugalisation. The object of these measures is to rid the "lymph" of the micro-organisms which accompany it. This object is, however, only imperfectly attained and is moreover accompanied by an attenuation of the vaccinal action. On the other hand, precautions are taken to ensure all possible cleanliness during the operation of inoculation and whilst the calves are under treatment. Thus, great care is taken to disinfect the area of inoculation with alcohol or some other antiseptic and to dress the pustules during the course of their development. Similarly the arms of the patient to be vaccinated are well washed ; following in this the rules of asepsis rather than of antisepsis for fear that the vaccinal virus might be destroyed by antiseptic substances. Various instruments are made use of for vaccination and care is taken to sterilise these before they are used. Sometimes the lancet is used, sometimes "plumes à vaccin" or vaccinostyles, or a bistoury of iridio-platinum (Lindenborn) etc.

When the vaccine is of good quality and the operation of vaccination is well done, there is no doubt as to the protective result obtained against small-pox. The observations that have been collected for a great number of years past, in many countries, place this beyond doubt. There are, indeed, statistics from which it is impossible to draw any precise conclusions because they are founded upon too scanty figures or deal with conditions that are too complex. This is the case with the Swiss vaccinations. Certain cantons (such as Zug and Uri) have made vaccination obligatory, whilst others (Bern, Zurich, Lucerne, etc.) some years ago abolished the law which compels the vaccination of all children in infancy. It happened that for some years small-pox had more victims in the cantons of the first group than in those of the second. The opponents of antivariolic vaccination attempted to use this as an argument against the utility of this method. But a more detailed study of the facts clearly shows that it is impossible to draw from it any conclusion whatever. Even in those cantons where vaccination is supposed to be compulsory this law is not carried out rigorously, and the number of persons vaccinated often does not exceed that in the cantons where it is not obligatory.

In order to gain some idea of the utility of vaccinations we must collect statistics on a much larger scale than are those obtainable from the Swiss cantons. Germany furnishes such statistics. Compulsory vaccination was introduced there more than a quarter

[480] of a century ago (1874), and statistical information has been collected with great care. With the exception of a slight increase during the period from 1879 to 1885 small-pox has diminished progressively since the proclamation of the new law, and has become so rare that in 1897 there were only 5 fatal cases in the whole German Empire. In the space of 13 years (1886—1898), in a population which embraces two-fifths of the total inhabitants of the German Empire, there were altogether five fatal cases of small-pox occurring in persons who had been successfully revaccinated. Moreover, the majority of the cases of small-pox occurred in the maritime towns or in the vicinity of the frontier of the Russian Empire.

Specially favourable results have been obtained in the German army, in which, even before the law of 1874, vaccination was compulsory. In 25 years there occurred in the Prussian army only two cases of death from small-pox. In summing up the statistical data on vaccination Kübler[1], from whom we have borrowed the above statements, expresses himself as follows: "The history of small-pox must in all cases register the fact that this dreaded disease has, as the result of general vaccination, not only become rare in the German Empire but that it has almost completely disappeared" (p. 365). The example of Germany encouraged several other countries to introduce compulsory vaccination, and Roumania, Hungary, and Italy have in turn promulgated similar laws. Here also it was not long before satisfactory results were obtained. In Italy especially the mortality from small-pox has largely decreased in recent years.

In England, where compulsory vaccination was introduced some time ago, it was abolished in 1898. As the opposition of the people became more manifest, the law, although it continued to exist formally, was carried out very imperfectly. The number of unvaccinated children had gradually increased in such a fashion that in London itself in 1897—1898 it attained the proportion of 24·9 %, whilst in certain provincial districts it has oscillated between 78·4 and 86·4 %. Under these conditions, the abolition of the law of compulsory vaccination was only the legal sanction of an accomplished fact. According to the details which have been supplied to me by the Jenner Institute in London (which has taken in hand the [481] distribution of vaccine), vaccinations since they are no longer compulsory have become more frequent in England, and the quantity

[1] "Die Geschichte der Pocken und der Impfung," von Coler's *Bibliothek*, Berlin, 1901.

of vaccine distributed has increased considerably. This quantity, however, is not adequate because small-pox has again made its appearance in London in the form of a pretty serious epidemic[1].

In France a law is being framed which will render infant vaccination compulsory. Up to the present this has not been the case, and small-pox from time to time causes considerable ravages, as we may see at this moment in Paris. During recent years the mortality from small-pox in France has been from 90 to 100 times greater than in Germany. It is greater amongst the female population than amongst males ; this constitutes a fresh argument in favour of vaccination. Although not compulsory for the whole of the French population, it is so for soldiers and for children who carry on their studies in schools, and it is for this reason that small-pox is rarer amongst males. The most complete demonstration of this is found in the incidence of small-pox in the French army. In spite of a less numerous contingent of troops (451,941—457,677) the mortality from small-pox was greater during the period when vaccination was not yet carried out generally (1885—1887) than during the period (1889—1896) when it was rigorously enforced on a much larger number of soldiers (524,733—564,643). From 13·6 fatal cases per year in the first period the annual figure fell to 6.

It follows, when we take into consideration the whole of the very numerous data at our disposal, that the usefulness of vaccination followed by revaccination after some (5—7) years cannot be seriously called in question. As to the inconveniences that may be caused, they are observed in very rare cases, and then most frequently when impure vaccines are used, or when the vaccinated skin becomes contaminated. According to the German statistics there were registered in the space of 13 years (1885—1897), in 32 millions of vaccinations, 113 fatal cases as the result of infection of the wounds. In forty-six of these it was proved that the small wound had been contaminated by impurities introduced by those attending on them. The remaining 67 fatal cases could be ascribed to the vaccines themselves. We must, however, still regard these cases as too numerous and as being readily avoidable by the adoption of rigorous asepsis. To sum up, the anti-variolous vaccination by the virus of cow-pox constitutes a method of very great value in the prevention of [482] one of the most dreaded of infective diseases, but it is evident that

[1] *Lancet*, London, 1901, Vol. II, p. 796.

improvement can still be made in this branch of practice. If science should succeed some day, as we may be permitted to hope it will, in finding the micro-organism of vaccinia and of small-pox, and it should succeed in growing it in pure media, it might react very beneficially on the practical application of vaccination. The more simple the methods, the less chance will there be of the occurrence of those unsuccessful cases which, even now, are rare exceptions.

II. *Vaccinations against sheep-pox* (*la clavelée*).—Sheep-pox, being a disease very similar to human small-pox and very serious from an economic point of view, the idea was conceived of fighting it by methods similar to those used against small-pox. Since the 18th century there has been practised on a large scale the artificial immunisation of sheep by the inoculation of the virus of the sheep-pox (clavelisation) just as the variolisation of man was practised before the discovery of cow-pox. For this purpose it was necessary to have a considerable quantity of virus; this was obtained by inoculating sheep-pox into the skin of sheep. This inoculation was effected either with a lancet or, according to Soulié's method[1], by means of a Pravaz syringe. The pustules, developed under these conditions, were generally of large size and capable of furnishing a considerable quantity of the virulent lymph (*claveau*) used for immunisation. This fluid, when gathered pure, and kept in a closed vessel protected from light and heat, retains its virulence for a long time: unlike what is observed in the case of vaccine, the addition of glycerine destroys the virulence of the lymph pretty quickly. For use, the lymph is diluted with ten times its volume of 2 % borated water; the fluid thus obtained is inoculated into the extremity of the tail or of the ear; usually a pustule, which remains single, is formed at the point of inoculation. Clavelisation rarely sets up a generalised eruption which is always serious and sometimes fatal.

In France the law ordains the clavelisation of flocks in which sheep-pox appears; but it interdicts its practice in unattacked flocks;—it is easy to understand the reason for this; in infected flocks all, or almost all, the sheep, gradually become ill and the
[483] illness lasts for some time; clavelisation diminishes both the duration and the gravity of the disease; the mortality that it causes, although sometimes very great, the French sheep being very susceptible to sheep-pox, is always much less than that due to a natural contagion;—

[1] *Médecine moderne*, Paris, 1896, p. 441.

on the other hand, the clavelisation of a healthy flock, beyond the fact that it may cause considerable losses, is attended by the special danger that it creates centres from which the contagion may invade all the flocks of the district.

But there are countries in which protective and general clavelisation does not present these inconveniences—the countries where the disease is endemic and where the sheep are very resistant to the action of its virus. This is the case in Algeria; sheep-pox exists there permanently without doing much damage; but the Algerian sheep, which take sheep-pox without suffering any apparent illness, communicate to French sheep amongst which they are introduced a very malignant sheep-pox which sometimes kills as many as 50 per cent. of the flock. This explains and justifies the measures recently taken by the Minister of Agriculture, forbidding the importation of Algerian sheep into France unless they have been vaccinated at least a month previously.

In many other countries clavelisation is likewise enacted, being authorised in cases where it may be very useful and interdicted in other cases. In certain countries, *e.g.* Germany, Holland, and Denmark, clavelisation can be put into force by the Government, which alone has the right to authorise it under certain circumstances.

III. *Antirabic vaccinations.* Vaccination against rabies has this point in common with those against small-pox and sheep-pox, that it is effected with a virus whose micro-organism is as yet unknown. On the other hand, it is distinguished by its efficacy during the incubation period. When persons are vaccinated during the incubation period of small-pox, or sheep during the same period of sheep-pox, the vaccinations by vaccine and *claveau* are incapable of arresting the disease and the infections continue to follow their normal course. When, on the other hand, we vaccinate men or animals that have been bitten by mad animals or inoculated with the rabic virus by other means, the antirabic vaccination, with rare exceptions, prevents the development of rabies. This vaccination, taking advantage of the length of the incubation period of rabies, constitutes, therefore, a special type, intermediate between protective vaccination, properly so called, and a therapeutic method of treatment. [484]

[1] Nocard et Leclainche, "Les maladies microbiennes des animaux," 2e édition, Paris, 1898, pp. 464, 469.

It is to Pasteur that science and humanity owe the invention of this method. Aided by his collaborators, especially by Roux, he established in the first place a whole series of important facts on the subject of the rabic virus and of experimental rabies. He then set himself to elaborate a practical method capable of preventing the manifestation of the disease in dogs inoculated with rabic virus and in men bitten by mad animals. He succeeded in solving this problem in 1885.

Pasteur's antirabic vaccines are prepared from the spinal cords of rabbits that have died of experimental rabies as the result of the inoculation of the virus bearing the name of "fixed virus." Prepared in the laboratory, this virus presents the characteristic feature that when inoculated under the dura mater of rabbits it sets up in them the first rabic manifestations after an incubation period of six or seven days. The disease soon assumes the typical paralytic form which lasts several days. Whilst the period of incubation presents only very limited variation, the time of death is subject to much greater variation, especially according to the season of the year. Sometimes the rabbits will die on the eighth day after the inoculation of the virus: but death may be delayed one or two days, rarely more.

It is necessary to wait for the natural death of the mad rabbits before the spinal cord is extracted, and not to kill them before this term, for it is only during the final moments of life that the rabic virus is abundant and is distributed uniformly through the whole substance of the organ. After removal from the vertebral canal the cord is suspended in glass vessels containing solid potassium hydrate at the bottom. A whole series of cords so prepared are then kept in a dark chamber heated to 23° C. or thereabouts. The progressive desiccation which the cords undergo under these conditions diminishes their virulence. At the end of several days of this treatment the desiccated cord, instead of producing rabies in 6—7 days in rabbits inoculated under the dura mater by trepanning, induces it after longer periods of incubation. Finally, the cords do not produce even the slightest symptoms of the disease.

The fundamental basis of the Pasteurian method consists in the fact that the desiccated cord, inoculated as an emulsion below the skin of animals, produces in them a complete and permanent
[485] immunity against inoculation of the most powerful rabic virus beneath the dura mater. This experiment, frequently repeated on

rabbits and dogs, justified Pasteur in 1885 in attempting the first vaccinations of persons bitten by rabid animals, especially dogs. The encouraging results of these early attempts led to the foundation of the Pasteur Institute in Paris, devoted, in part, to antirabic vaccinations. Shortly afterwards, antirabic Institutes were founded in many other European towns, and later in North and South America, in Indo-China, the East Indies, and in Africa. At present there are in France six such Institutes (Paris, Lille, Marseilles, Montpellier, Lyons, Bordeaux), in Russia 9, in Italy 6, etc. The last of these institutions founded in Europe is that of Berlin, where it forms a branch of the Institute for Infective Diseases carried on under the direction of Robert Koch. The foundation of an antirabic institute in Berlin had a very important significance from several points of view. In the first place, it indicates the definite acceptance of the Pasteurian method, a method which has been discussed so long and so keenly. Secondly, it proves that even in a State where there is a highly organised sanitary police, antirabic vaccinations may still be of great service.

Seeing that it was in the Pasteur Institute of Paris that the method of antirabic vaccinations was first elaborated and that it has undergone a very prolonged ordeal, the method there used serves as a model for the practice of almost all other institutes. Although in some of them methods which differ more or less from the original may have been introduced, the fundamental principle upon which they are based remains the same.

According to the Pasteurian method properly so called the vaccinal inoculations are commenced with cords that have been dried for 14 days and have thus lost their virulence. A piece five millimetres long is pounded up with very weak veal broth. Up to 3 c.c. of the emulsion thus prepared is injected below the skin of the flank. The same day a second injection of the same quantity of an emulsion of a cord which has been drying for 13 days is made at the corresponding position on the opposite side. Each day an advance is made by injecting emulsions of cord which are increasingly fresh and the treatment is concluded by the introduction of virulent cords, which have been kept at 23° C. for 3 days only. The ordinary medium treatment lasts for 15 days. On the first 5 days two vaccine injections a day are made. On the last 10 days, when gradually fresher and more virulent cords are employed, only a single in- [486]
jection is made each day. The injections are made with syringes of

the Pravaz type and are carried out under conditions of rigorous cleanliness.

If the bites are numerous, or if they are situated on exposed parts, the treatment is prolonged for 18 days and is further distinguished in that the cords of 4 and of 3 days are injected much more frequently.

In especially grave cases, when the bites are on the face and head, the treatment extends over 3 weeks. A more rapid progress is made by making four injections instead of two during the two first days; in this way a greater quantity of the virulent cords is injected than in the first two types of treatment.

The effect of the antirabic vaccinations is usually very good. During the early years of their application the results were fully discussed from all points of view, and no efforts were neglected of seeking out objections of every kind. For the purpose of obtaining rigorously accurate statistics a separate division was made, at the Pasteur Institute, for the cases of persons treated after bites inflicted by dogs whose rabic condition had been demonstrated experimentally (by the injection of an emulsion of the bulb below the dura mater or into the anterior chamber of the eye of the rabbit or guinea-pig). A second and special set of statistics was drawn up of cases where the bites had been inflicted by animals whose rabic condition had been recognised by veterinary examination. Individuals bitten by animals that were simply suspected to suffer from rabies were kept separate.

Thanks to this systematic classification we were able, at the Pasteur Institute of Paris, to establish the fact that the antirabic vaccinations performed on persons bitten by animals that were undoubtedly mad resulted in an extremely low mortality from rabies. Finding it impossible to attack these results, demonstrated with the precision of a laboratory experiment, the adversaries of the Pasteurian method alleged that, quite apart from any vaccination, the percentage of cases of rabies in persons bitten by mad animals is not greater than amongst the vaccinated. A hitch in the application of the new vaccinal method soon demonstrated how entirely unfounded was this objection. At the Bacteriological Institute of Odessa, founded in 1886, that is to say almost immediately after the Paris Institute, the first attempts at vaccination were followed by a mortality from rabies of 5·88 per cent., a figure incomparably higher than that of the Paris Institute. Analysing the probable causes of
[487] this want of success it was found that the Russian rabbits, being

much smaller than the French ones, furnished far too small an amount of vaccinal matter. This being the case, the introduction of a more intensive treatment was sufficient to cause the mortality to drop suddenly to 0·8 per cent. This fact, added to so many other proofs, finally convinced the most sceptical and brought about a general acceptance of the Pasteurian method.

In course of time the number of cases observed has become very considerable and the experience gained in the manipulation of this method very wide. The improvements made in the details of the vaccinal practice have brought about a progressive diminution in the mortality amongst the persons treated. From 0·94 per cent. in 1886 the mortality (counted from the 16th day after the completion of the vaccinations) fell in 1897 to 0·39 per cent., in 1900 to 0·28 per cent. In the space of 15 years (1886—1900) there have been treated in Paris 24,665 persons, of whom 107 died from rabies, giving an average of 0·43 per cent.[1]. The greatest mortality was registered during the early years of the application of the method, and the rate of the later years (1896—1900) oscillated between 0·39 per cent. and 0·20 per cent.

The results obtained in the majority of the other antirabic institutes corroborate those of the Pasteur Institute of Paris. Thus, according to the latest statistics of the St Petersburg Institute[2], the mortality, in 1899, among persons who had completed their vaccinations, was about 0·5 per cent. At Berlin[3] there were treated during the same period 384 persons, of whom 2 died from rabies during treatment, whilst a third succumbed on the 14th day after the close of the vaccinations. Only this latter case ought, according to the principles generally accepted, to be counted as an unsuccessful case, this would give a mortality of 0·26 per cent.

Quite recently, the antirabic treatment has been so reinforced that the treatment terminates with the injection of cords desiccated for two days or even one day only. The results of this intensive treatment have not yet been reported upon.

According to the statistics of the Berlin Institute rabies is far

[1] Report by Viala in the *Ann. de l'Inst. Pasteur*, Paris, 1901, t. xv, p. 445. There will be found in Marie's work, "La rage" (*Collection des aides-mém.*, Paris, 1900), many details on antirabic vaccination.

[2] According to Krajouchkine, in the *Arch. d. Sci. biol.*, St Pétersbourg, 1901, t. viii, p. 349.

[3] According to Marx in *Klin. Jahrb.*, Berlin, 1900, Bd. vii, S. 1.

[488] from being so rare in Germany as was, at one time, generally supposed. During the year 1899 its presence was demonstrated, by the experimental method, in 206 dogs coming from various districts. It is in Silesia, Western Prussia, and Posen that rabies in dogs has been observed most frequently.

Antirabic vaccinations have also been performed on herbivorous animals (sheep, goats, cattle, and horses) which are immunised by means of injections of the rabic virus into the veins, according to the method suggested by Nocard and Roux[1], as the result of experiments made by Galtier[2].

IV. *Vaccinations against rinderpest.* For some time attempts were made to find a means of immunising the Bovidae and other ruminants, susceptible to rinderpest, against this terrible disease, which causes great ravages in regions where it is endemic and greater still in those regions where it only appears in epidemic form. The good results obtained from "clavelisation" suggested the idea of immunising against rinderpest by the inoculation of the rinderpest virus, but all such attempts gave unsatisfactory results, the inoculation setting up a rinderpest as grave, and often as fatal as the natural disease. Only in recent years have we succeeded in elaborating methods of vaccination really capable of coping effectively with rinderpest. Koch[3] went to Cape Colony, where this disease had recently appeared and had caused enormous losses, with the intention of finding a practical method of arresting the scourge. In spite of his technique and incomparable skill he was as unsuccessful in finding the parasite of rinderpest as had been other investigators. The micro-organism of this disease remains unknown. It was necessary, however, to seek a remedy against it. Koch, studying the properties of the bile of animals that had died from rinderpest, recognised that the injection of this bile into normal animals conferred upon them a fairly certain immunity, and this fact served as the basis on which to work out a practical method of combating rinderpest on a large scale. At first this method was received with
[489] much enthusiasm, but experience soon demonstrated the inconveniences it often presented. Kolle and Turner[4], who continued the

[1] *Ann. de l'Inst. Pasteur*, Paris, 1888, t. II, p. 341.
[2] *Compt. rend. Acad. d. sc.*, Paris, 1881, t. XCIII, p. 284.
[3] *Deutsche med. Wchnschr.*, Leipzig, 1897, SS. 225, 241.
[4] *Ztschr. f. Hyg.*, Leipzig, 1898, Bd. XXIX, S. 309.

researches on rinderpest in Cape Colony, extolled Koch's method at the commencement of the epidemic with the object of establishing around the original disease centre an unaffected zone which would interfere with the propagation of the disease. They recognised, however, that this method could not be employed generally, for the reason that it does not set up immunity until the end of eight days, during which period the animals may contract the disease. Further, it demands the sacrifice of a large number of animals in order to provide the vaccinal bile required for the vaccinations ; finally, it confers an immunity of short duration only (four to six months).

It was necessary, therefore, to find some method that was more generally applicable. With this object Koch himself began to study the blood serum of animals that had recovered spontaneously from rinderpest. He was able to assure not only himself, but several other observers, that this serum was capable of rendering normal animals into which it is injected refractory. Bordet and Danysz, who studied rinderpest in the Transvaal in 1897, made many experiments in this direction and devised a method which gave good results in practice. But it was left to Kolle and Turner to work out a method at once simple and easily applied, one which soon came into general use. This method is known by the name of "simultaneous vaccinations." It consists in the injection of a protective serum simultaneously with the virulent blood. To prepare the former the authors just mentioned made use of animals that had recovered spontaneously from rinderpest or of Bovidae that had been immunised by bile or by some other method. It was recognised that the protective power of the serum of animals that have recovered is very small and cannot confer immunity on normal animals, except when injected in large doses. Kolle and Turner showed that if Bovidae that have recovered spontaneously are injected with very large quantities of virulent blood coming from animals fatally attacked, the protective power of the serum of the former is markedly increased and a serum is obtained which is active in small doses and which gives good results in practice. This serum may be kept for a long time by the addition of a small quantity of carbolic acid. The immunity conferred by this serum upon normal animals is immediate, but of short duration; it [490]
is completed by making a simultaneous injection of virulent blood ; we thus obtain a double immunity, one part immediate, the other permanent; to get this result, however, the serum must not be mixed with the virulent blood, for when this is done the immunity conferred

is trifling or *nil.* On the other hand, it is complete and persists for several months when the protective serum is injected separately on one side of the body and the virulent blood on the other.

Kolle and Turner had to defend their method against many ill-founded objections and attacks, but they succeeded in getting it accepted, not only in Cape Colony but also in many other parts of Africa, and in many countries in Europe and in Asia. In 1898 it was decided at a conference which met in Cape Town to use the method of simultaneous vaccinations to the exclusion of all others. This method has since been applied on a very large scale and it was not long before favourable results were obtained. The same method has proved to be very successful with Nicolle and Adil-Bey[1] of Constantinople, who now prepare large quantities of the antirinderpest serum, and combat this disease with great success in the Ottoman empire. Yersin[2] adopted the same method to fight the cattle plague in Indo-China, where it causes great ravages, especially among buffaloes. His Institute at Nha-Trang has become a centre for the preparation of the specific serum, which he distributes over a vast territory. In the East Indies the simultaneous method has been applied by Rogers[3]. In Russia, where rinderpest is endemic in many regions, the Institute of Experimental Medicine at St Petersburg furnishes the serum destined to prevent the propagation of this epizootic disease[4].

In a few years this method of simultaneous vaccination has been extended to all the countries ravaged by rinderpest and has already rendered immense services to agriculture.

V. *Anti-anthrax vaccinations.* In the first four sections of this Chapter we have brought together the methods which have as their
[491] basis the vaccination by viruses whose nature is as yet unknown. Since we cannot obtain them by artificial culture, we have to introduce them with animal fluids:—either the contents of vaccinal or clavelar pustules, or matter from rabic nervous centres, or again the blood of animals attacked by rinderpest. In the case last mentioned, in order to prevent the too serious effect of the injection of the virus, it is combined with a simultaneous injection of protective serum.

[1] *Ann. de l'Inst. Pasteur*, Paris, 1899, t. XIII, p. 319; 1901, t. XV, p. 715.

[2] *Rec. de méd. vét.*, Paris, 1901, pp. 48, 115.

[3] *Report on an experim. Investig. of the method of Inoculation against Rinderpest*, Calcutta, 1900; *Ztschr. f. Hyg.*, Leipzig, 1900, Bd. XXXV, S. 59.

[4] Nencki, Sieber and Wyznikiewicz, *Arch. internat. de Pharmacodyn.*, Gand et Paris, 1899, vol. V, p. 475.

In the case of the vaccinations against anthrax we pass to the group of viruses whose organised nature is well known and which can be injected in pure culture grown on artificially prepared media. This method constitutes one of Pasteur's most brilliant discoveries, made in collaboration with Chamberland and Roux. Before they had found a satisfactory method of vaccinating against anthrax these observers had to solve the problem in connection with a less complicated and less difficult case. From the first, in his studies on pathogenic micro-organisms, Pasteur had devoted his attention to finding a means of communicating immunity against these parasites. With the aid of Chamberland and Roux he was not long in discovering a method by which it was possible to attenuate the virulence of the micro-organism of fowl cholera and to vaccinate fowls against this terrible disease by inoculating them with this attenuated micro-organism. Guided by these results Pasteur, Chamberland and Roux set to work to find the vaccine against anthrax; they were soon confronted by a serious obstacle in the formation of spores which prevented the attenuation of the bacilli. This obstacle they overcame by submitting cultures of the bacillus to a temperature of 42·5° C. Under this condition spores do not develop, and the bacilli become attenuated at the end of a longer or shorter period. Although in possession of these attenuated viruses, it still needed very laborious investigations to adapt them to the vaccination of various species of animals susceptible to anthrax, especially sheep. In this they were also successful, and in 1881, over 20 years ago, Pasteur and his collaborators demonstrated the efficacy of their method on a large number of animals. This demonstration was made at Pouilly-le-Fort before a large commission. We may affirm that this celebrated experiment opened a new path to science and to the practice of vaccination. It was performed on 50 sheep, half of which were vaccinated twice with twelve days' interval, the other 25 sheep serving as control animals. Fourteen days after the vaccination by the second vaccine [492] all the 50 sheep were subjected to a test inoculation of a very strong anthrax virus. Two days later the vaccinated animals remained unaffected, whilst the control animals had all succumbed to anthrax.

Similar experiments, undertaken in France, Hungary, Germany, Russia and elsewhere, confirmed the efficacy of anthrax vaccinations and led to their extension into all the countries where bacterial anthrax was rife. From the year 1881 the method came into regular use, and before the end of that year there had been vaccinated, in

France alone, 62,000 sheep and 6,000 Bovidae. Since these first attempts, made on a large scale, gave such good results, the anti-anthrax practice was not long in spreading through France, then into Hungary and several other European countries. Later, it extended into other continents, especially into South America (Argentina)[1] and Australia. Vaccinations against anthrax were also applied to horses with the same good results[2].

In France the anti-anthrax vaccines are prepared at and sent out from the Pasteur Institute of Paris. These vaccines consist of broth cultures of attenuated bacilli, of which the weakest, the first vaccine, is fatal to the mouse and small guinea-pigs. The bacilli of the second vaccine are less attenuated, and are capable of killing not only adult guinea-pigs but even a certain number of rabbits, when inoculated subcutaneously. The two vaccines are races of the anthrax bacillus, capable of producing spores which present the same degree of virulence as the filamentous bacilli which gave them birth.

The anti-anthrax vaccines are sent out in tubes containing the quantity necessary for the vaccination of a large number of animals. The vaccinations are made especially in spring in order that the animals may be protected during the hot season, which is usually more favourable to the development of anthrax epidemics.

In the sheep the vaccines are injected below the skin on the inner aspect of the thigh. One-eighth of a c.c. of the first vaccine is injected with a somewhat modified Pravaz syringe. Twelve or
[493] fifteen days later a similar injection is made on the opposite side with the second vaccine. In the Bovidae the vaccines are injected behind the shoulders, where the skin is thinnest. In the horse the injections must be made on the sides of the neck and shoulders. In large mammals double the amount ($\frac{1}{4}$th of a c.c.) of each vaccine is injected.

The tubes of vaccine, once opened, should not be employed a second time. Care must be taken to use the whole of their contents at one series of vaccinations.

The vaccinal injections produce tumefaction at the point of inoculation and are followed by a slight rise of temperature. But these symptoms are of little importance and soon disappear. Serious complications and fatal results from the vaccinations are very rare.

[1] J. Mendez, *Anal. d. Circ. Med. Argent.*, Buenos Aires, 1901, t. XXIV, Nos. 5, 6.

[2] On the methods of vaccination against anthrax see Chamberland, "Le charbon et la vaccination charbonneuse," Paris, 1883.

The loss due to these accidents is estimated at one-half per cent. in sheep and a quarter per cent. in the Bovidae.

The refractory condition resulting from the vaccination requires for its development a period of about a fortnight. The immunity is then very substantial and lasts for a fairly long time. According to Chamberland 60 % of the sheep retain their immunity a year after they have been vaccinated. But as a great number of animals then become susceptible, it is usual to revaccinate annually.

According to the statistics furnished by the vaccine department of the Pasteur Institute there have been vaccinated, up to the 1st of January 1900, a total of 4,971,494 sheep, and 708,980 cattle. Abroad the corresponding figures are 3,831,948 and 1,869,445. Altogether, the number of animals vaccinated amounted to 11,381,867, of which 3,626,206 have been treated with the vaccine furnished by the Budapest Laboratory.

The results of the anti-anthrax vaccinations were found to be so favourable that it was unnecessary to introduce any improvements in technique. Attempts have certainly been made to prepare anti-anthrax serums, and these have been successful, but up to the present such serums have not been introduced into practice.

VI. *Vaccinations against symptomatic anthrax.* Symptomatic anthrax, which is often confounded with true anthrax, is set up, as demonstrated by Arloing, Cornevin, and Thomas, by a specific anaerobic micro-organism to which has been given the name of *Bacillus chauvaei.* Immediately after the discovery of the attenuation of [494] viruses and of vaccines against fowl cholera, the three observers above mentioned tried to apply it to symptomatic anthrax. Finally they devised a method which was soon adopted in practice, and which, for nearly twenty years, has been used in the vaccination of the Bovidae in countries where symptomatic anthrax is most prevalent. This is especially the case in mountainous districts, such as Switzerland, the Bavarian Alps, the Dauphiné, L'Auvergne, etc.

Arloing, Cornevin, and Thomas[1] prepare two vaccines against symptomatic anthrax by a method very different from that used in the preparation of the Pasteurian anti-anthrax vaccines. They take the virus from the muscles invaded by the micro-organism ; they triturate a piece of the tumefied muscle in a mortar, adding to it a few

[1] "Le charbon bactérien," Paris, 1883 ; 2e édition, 1887.

drops of water. The mixture is filtered through muslin and the fluid dried at 37° C.; a virulent brown powder is thus obtained. In the preparation of the vaccines a portion of this powder is mixed with water and subjected to a temperature of 100°—104° C. for seven hours. Another portion is heated during the same number of hours to 90°—94° C. only. This latter forms the second vaccine whilst the first portion constitutes the first.

In practice the two vaccinal powders are dissolved in cooled boiled water and are introduced into the subcutaneous tissue of the animals that it is wished to immunise. The second vaccine should be injected 8 to 12 days after the first. The vaccines are usually tolerated very well by the Bovidae and confer upon them a definite and permanent immunity. In spite of certain drawbacks this method, known as the "Lyons method," has proved to be a very serviceable one and is retained as the best devised up to the present. Its efficacy is proved by the fact that in the period from 1884 to 1895 in 400,000 vaccinated animals the mortality has only been 1 per 1,000. Arloing, Cornevin, and Thomas thought that raising the virus to a high temperature brought about a real attenuation.

Leclainche and Vallée[1], who have recently returned to the study of this question, have shown that this view cannot be maintained.
[495] In reality the spores, after being heated to 90°—104° C., gave rise to bacilli endowed with their normal and complete virulence. But the heating in the preparation of the Lyons vaccines destroys the toxin manufactured by the *Bacillus chauvaei*, with the result, that the spores now become the prey of phagocytes: it is for this reason and for this reason alone that the inoculation of these vaccines is so well tolerated. All the spores of the vaccinal powder are not eaten by the phagocytes: those which are found in the centre of solid particles of the powder offer a prolonged resistance to the action of the cells, and some of them germinating produce bacilli and give rise to a mild disease capable of conferring immunity. The germination of these spores is further facilitated by the presence of foreign micro-organisms in the vaccinal powders; these organisms help to interfere with the phagocytosis of the spores of symptomatic anthrax.

In the course of their researches, Leclainche and Vallée demonstrated that it is easy to vaccinate animals susceptible to anthrax and to confer on them a substantial immunity by means of a single protective injection of a pure culture of *Bacillus chauvaei*. For this

[1] *Ann. de l'Inst. Pasteur*, Paris, 1900, t. XIV, pp. 202, 513.

purpose they use cultures grown in broth made from the pig's stomach ("bouillon de panse" or Martin's broth) which they heat for 2 hours at 70° C. The cultures, so treated and injected in quantities of 1 to 2 c.c. into Bovidae, induce in them an immediate immunity. These authors are persuaded that the vaccination by this method might be used on a large scale with certain advantages over the method at present in use. A single injection, instead of two, involves a great economy, and the injection of pure vaccinal cultures obviates the accidents caused by the foreign organisms which are found mixed with the Lyons vaccine.

On the other hand, Leclainche and Vallée think that vaccination by serums has no future in the fight against symptomatic anthrax and should only be used in exceptional cases.

It is evident that the Lyons method is capable of being improved and some day may be replaced by another. Still it must be remembered that it has already preserved a very great number of animals from certain death by symptomatic anthrax.

VII. *Vaccinations against swine erysipelas.* Swine erysipelas is a disease widely distributed in nearly all countries where the breeding of pigs is carried on on a large scale. It is a very fatal disease, and it is estimated that in France alone at least 100,000 pigs of the value of more than five million francs succumb to it annually. Unfortunately [496] swine erysipelas is often confounded by breeders with other epizootic diseases, especially pneumo-enteritis of the pig. This confusion has often resulted in large losses to agriculture.

Soon after the vaccinations against anthrax became a part of veterinary practice, Pasteur[1], assisted by Thuillier, took up the study of swine erysipelas which was causing great ravages in the department of Vaucluse. They were not long in discovering that the true cause of the disease was a very small bacillus capable of growing in pure culture in nutrient broth. Guided by his former investigations, Pasteur with his collaborator undertook minute researches into the reinforcement and attenuation of the virulence of the bacillus of swine erysipelas which led them to the elaboration of a method of vaccination capable of conferring on pigs a high degree of protection against the disease. Following the line of the anthrax vaccinations, Pasteur and Thuillier prepared two vaccines against the erysipelas, the first more attenuated than the second. The bacilli of these two vaccines

[1] *Compt. rend. Acad. d. sc.*, Paris, 1883, t. XCVII, p. 1163.

were cultivated in broth and sent out in tubes similar to those employed in the distribution of the anthrax vaccines.

The vaccines are in themselves innocuous and are capable of communicating to the inoculated pig an immunity sufficiently durable to be of real service. Young pigs being less susceptible to the erysipelas than are the adults, it is generally preferred to vaccinate young pigs of from two to four months. The vaccination is done at two separate times. The first vaccine, in a dose of one-eighth of a cubic centimetre, is inoculated subcutaneously on the inner aspect of the right thigh ; the second vaccine is inoculated in the same way, 12 or 15 days later, into the left thigh. The immunity that follows these vaccinations is not fully established until the end of the second week.

In spite of the many advantages of the Pasteurian method the vaccinations against swine erysipelas have not spread so much as one might have expected ; and they have found a general application abroad rather than in France. It is only necessary to cast a glance at the statistics to be convinced of this. From the date of the introduction of the Pasteurian vaccinations in 1884 up to the 1st January, 1900, there had been vaccinated in France in all 428,746 pigs, whilst abroad, where the vaccinations were introduced some
[497] years later, the number of pigs vaccinated was 4,819,387. Of this number the great majority (4,194,191) had been treated in Hungary. The losses amongst the vaccinated animals were insignificant (1·68 %) when compared with an average mortality of 20 % amongst unvaccinated pigs.

This limited extension of the vaccination of pigs in France arises from various causes. In many countries the breeding is on too small a scale to allow of the intervention of the veterinarian and of the expenses which the vaccinations involve. On the other hand, it cannot be denied that the Pasteurian method presents certain drawbacks in practice. The living, although attenuated, bacilli introduced may sometimes serve as centres of infection, especially in cases, rare no doubt, where the vaccinated animal contracts a chronic form of the disease. The Pasteurian vaccines must, therefore, be avoided in districts where the erysipelas has not yet appeared. Their application in countries already infected presents the further drawback that the immunity requires for its establishment a fairly long time, sufficiently long to permit the micro-organism to kill a large number of pigs before the vaccines have conferred any immunity upon them.

It is natural that, under such conditions, an attempt has been

made to replace the Pasteurian method by some other method less risky. Hence, since the discovery of the principle of sero-therapy several investigators have sought to apply it to swine erysipelas. Emmerich and Mastbaum[1] were the first to demonstrate that the blood of rabbits, immunised with the bacilli of this disease, acquire a very marked protective power. They have even attempted to construct from the results of their researches methods which might be applied practically. It is especially however to Lorenz[2], a Darmstadt veterinarian, that we owe the first practical application of this method. He prepared protective serums by injecting erysipelas bacilli into rabbits and pigs, and demonstrated that the inoculation of these serums, when combined with that of the living bacilli, conferred upon pigs a sufficient immunity and one that was set up immediately after the introduction of the serum. According to Lorenz's method it is first necessary to give a protective injection of serum; some days (3—5) afterwards this is followed by an inoculation of living bacilli coming from the attenuated erysipelas known in Germany under the name of [498]
"Backsteinblattern." About two weeks later a further injection of the same bacilli, but in double quantity, is given. This method, therefore, involves three vaccinal injections as against two in the Pasteurian method. It is consequently dearer than the latter, but, as it presents certain undeniable advantages, an attempt was made to introduce it into veterinary practice. But being much more complicated endeavours were made to simplify it. Voges and Schütz, by methods which have remained secret, soon obtained a more active serum, and finally Leclainche[3] of Toulouse, after demonstrating that the horse is the best animal for the production of a very active serum, succeeded in devising a method of vaccination as simple as it was effective. He gave to it the name of "serum-vaccinations." The first inoculation is made with a mixture of specific serum and a culture of living and virulent bacilli. This inoculation is well borne by all pigs and may be made without any regard to the age of the animal. The immunity is set up immediately after the injection of the mixture, but it is not sufficiently durable for the requirements of practice. For this reason Leclainche followed up the first injection by a second, which

[1] *Arch. f. Hyg.*, München u. Leipzig, 1891, Bd. XII, S. 275.

[2] *Deutsche thierärztl. Wchnschr.*, Karlsruhe, 1893, Bd. I, SS. 41, 85; *Centralbl. f. Bakteriol. u. Parasitenk.*, Jena, 1893, Bd. XIII, S. 357; *Deutsche Ztschr. f. Thiermed.*, Leipzig, 1894, Bd. XX, S. 1.

[3] *Rev. vét.*, Toulouse, 1900, t. LVII, p. 346.

is made ten to twelve days later and consists of an inoculation of half a cubic centimetre of pure virus. This new method had the special advantage of arresting, almost immediately, the mortality in an infected piggery and of eliminating the chronic cases that are sometimes observed after the Pasteurian vaccinations.

Leclainche[1] has already applied his method of serum vaccinations to more than five million pigs of all ages. "It has been found to be constant in its effect and absolutely innocuous," and "not a single case of erysipelas has been met with in pigs that had received the two vaccines," and Leclainche hopes that his method will soon come into general practice, and that it will be utilised in all cases where the Pasteurian method is found to be insufficient.

As the basis of all the new methods for vaccinating pigs against erysipelas is the preparation of serums capable of preventing the pathogenic effect of the bacilli, the question of the determination of the protective power of these serums comes to be one of considerable importance. At first one was satisfied with certain approximate
[499] estimations, but later the necessity was felt of having a more exact measurement. Leclainche is persuaded that of all the laboratory animals capable of being used for these experiments the pigeon is the only one that can usefully fulfil this rôle; very susceptible to the passage virus, it is killed by the bacillus after a regular incubation and invasion period, and the chronic form of the erysipelas, so troublesome in the rabbit and even in the pig, is met with in the pigeon in very exceptional cases only. Leclainche commenced his experiments by inoculating into the pectoral muscles of the pigeon mixtures of serum and virulent cultures. The pigeon received 1 c.c. of a culture of a passage virus mixed with variable quantities of serum. The serum is ready for use in the vaccination of pigs when the pigeons resist the injection of a mixture of $\frac{1}{2}$ a c.c. of serum with 1 c.c. of a virus which kills the control pigeons in 60 to 72 hours.

At the Frankfort Institute of Experimental Therapeutics another method of testing devised by Marx[2] is used. In it injections, below the skin of a series of grey mice, are made of progressively increasing doses of the serum the strength of which it is desired to determine. Twenty-four hours later a virulent culture of the bacillus of swine erysipelas is introduced into the peritoneal cavity of the same mice. The virus is so chosen that the control mice die in about 72 hours.

[1] *Rev. vét.*, Toulouse, 1901, t. LVIII, p. 149.
[2] *Deutsche thierärztl. Wchnschr.*, Karlsruhe, 1901, No. 6.

Marx finds that this method gives results which are much more constant and exact than any other; this opinion is confirmed at Höchst, the largest factory of serums in Germany.

VIII. *Vaccinations against bovine pleuropneumonia.* This infective disease is one of the most dreaded scourges of bovine animals. Very contagious, it has spread from central Europe not only into all the other countries of the European continent, but into Africa, America, and almost every quarter of the globe. The virus of this disease was discovered in the serous exudation of hepatised lungs long before the microbiological period of the Medical Sciences had begun.

Dr Willems of Harselt, who made an experimental investigation, remarkable for the time at which it was carried out (more than half a century ago), demonstrated at once the great virulence of the pulmonary serous fluid; he found also that the effects of the inoculation of the virus varied much according to the seat of inoculation. When [500] made into the trunk, the neck, or the shoulders, the inoculations are usually fatal; at the periphery, the lower part of the limbs, at the extremity of the ears or of the tail, the inoculation ordinarily produces merely an inflammatory tumefaction of small extent, which is absorbed in a few weeks; after this the animal is refractory to the natural disease. Willems concluded from this that we may vaccinate against pleuropneumonia by inoculating the virulent serous fluid of the lung into the tail. Willems' method of inoculation became a part of current practice 50 years ago.

For the carrying out of a large number of vaccinations it is necessary to have at one's disposal an adequate quantity of virus; it was therefore to meet this requirement that researches were first carried out. The serous fluid was withdrawn from the hepatised lungs of animals that had succumbed to the disease and was inoculated into normal Bovidae as soon as possible, so as to avoid contamination of the fluid. In fact this pulmonary serous fluid often contains foreign germs capable of multiplying rapidly so that it putrefies very quickly. Pasteur showed that it was possible to remedy these drawbacks by a very simple method by which he could obtain a large quantity of rigorously pure virus. All that is necessary is to inoculate a little of the pleuropneumonic virus below the skin of a weaned calf, behind the shoulder. At the seat of inoculation there is an abundant exudation of virulent serous fluid into the cellular

tissue, from which we are enabled to collect large quantities of pure virus.

In some countries, as in Germany and in Australia, institutions have been founded for the production by this method of the virulent serous fluid necessary for these inoculations.

The virus should be inoculated into the tip of the tail of animals that it is desired to immunise, because the temperature in this situation is relatively low and the connective tissue is dense and not very abundant. The inoculation is made with a lancet or a Pravaz syringe. The vaccination is generally borne well, in spite of the reaction phenomena which are manifested about two weeks after the introduction of the virus. At that time a febrile condition is set up and a swelling manifests itself at the point of inoculation, which, however, soon retrogresses and then disappears.

The immunity conferred by Willems' method is substantial and lasting (for one or two years and even longer); this explains its great success in the hands of breeders and veterinarians. Accidents fol-
[501] lowing its use are rare, and the mortality does not exceed 1 per cent.

In spite of all these advantages a new method was still desirable, a method which would allow of the preparation of large quantities of virus of a suitable and uniform activity under conditions of irreproachable purity. Thanks to the discovery of the micro-organism of pleuropneumonia which we owe to Nocard and Roux[1] this object has been achieved. With the collaboration of Borrel, Salimbeni, and Dujardin-Beaumetz, they succeeded in demonstrating and isolating this micro-organism, the smallest of all known living organisms. The first steps in these researches were very laborious, but later the organism of pleuropneumonia was cultivated on fluid and solid media: Martin's broth (prepared with pigs' stomachs) or agar with the addition of a certain quantity (about 5 %) of fresh ox serum. The serum-broth, sown with pure pneumonic serous fluid, gives only a moderate growth, which becomes only slightly turbid and contains micro-organisms so small that it is impossible to distinguish them individually. They can be made out only when massed together in irregular clumps. The minuteness of this micro-organism is evidenced by the ease with which it passes through a Berkefeld filter, and even through certain Chamberland candles (F). This feature enables us to

[1] *Ann. de l'Inst. Pasteur*, Paris, 1898, t. XII, p. 240; *Cinquanten. d. l. Soc. d. biol.*, Paris, 1899, p. 440; Dujardin-Beaumetz, "Le microbe de la péripneumonie," Thèse de Paris, 1900.

obtain the pure virus easily, a fact very important in connection with the isolation of the micro-organism.

Once in possession of pure cultures of the micro-organism of pleuro-pneumonia, Nocard and Roux attempted to make use of it in practical vaccination. They showed that the organism separated by them is capable of producing typical pleuropneumonia when it is inoculated into the appropriate regions of the body of bovine animals. But when inoculated subcutaneously or into the skin of the tail, it produces merely a mild and transient disease which confers an immunity quite as effectual as that set up by the inoculation of the virulent serous fluid. It may be readily understood that, under these conditions, pure cultures may be much more serviceably employed in the practice of vaccination than can Willems' virus from the fact that it is easy to obtain large quantities of absolutely pure cultures. It is easy to predict that the new method will soon replace the old one, very great as are the services the latter has rendered to agriculture. [502] Up to the present, vaccinations with pure cultures have been made in several districts in France with very favourable results. The Pasteur Institute and the Veterinary School at Alfort have already distributed to veterinary surgeons more than 5,000 vaccinal doses of culture ; the protective action of these inoculations has been at least equal to that of the inoculations by Willems' method and the resulting accidents have been reduced in the proportion of 20 to 1[1].

The serum of animals hyperimmunised against pleuropneumonia possesses a very distinct *protective* action, but too little marked and of too short duration to be of any use in practice ; it has also a *curative* action arresting the invading march of a pleuropneumonic congestion ; but here it is necessary to intervene early, before the appearance of fever, and to inject large quantities of serum.

The inoculation of a mixture of virus and serum produces no congestion ; but it does not confer any immunity ; the animal remains just as susceptible as the control to the inoculation of the pure virus.

IX. *Vaccinations against typhoid fever.* In the preceding sections I have treated more especially of the vaccination of domestic animals against several infective diseases. The information collected

[1] In 1884, in the Department of the Basses-Pyrenées, the Willems' method of inoculation was carried out on 1354 Bovidae ; of this number 10 died and 45 lost their tails completely. In 1901, in the same department, 2800 Bovidae were inoculated with pure cultures, only 1 died and 9 lost their tails.

on this subject is marked by its great exactness, as it is easy to apply to animals the most rigorous experimental method. In the case of the human subject this is not such an easy matter. As it is impossible to submit him to experimental proof we are obliged to be satisfied with observation, controlled by statistical data. The experience of more than 100 years has, however, been sufficient to demonstrate the great utility of vaccinations against small-pox with the virus of cow-pox which is innocuous for the human subject. In the case of antirabic vaccinations we have to deal with injections into the human subject, first of weakened viruses and then of virulent viruses. Here, however, it is a question of the preservation of the already infected human organism, which, very often, only comes under treatment during the incubation stage of rabies. One can readily understand the hesitation to inoculate even weakened viruses into the human subject, especially
[503] when we are not dealing with altogether exceptional cases such as we have in the protection against rabies. We have, therefore, but few examples in which the methods of vaccination by micro-organisms have been applied to man. Such injections were first tried by Ferran[1] against Asiatic cholera. Having succeeded in vaccinating guinea-pigs against experimental cholera septicaemia, the Spanish investigator attempted to inoculate cholera vibrios into the subcutaneous tissue of man, hoping thus to vaccinate him against true cholera. In this way he was able to demonstrate that the subcutaneous injection of living vibrios never sets up symptoms of cholera. The injection is followed by a general reaction in the form of fever, pains in the back and inflammation at the point of inoculation, in a word, transient phenomena of little gravity. Encouraged by these initial results Ferran, profiting by the outbreak of cholera in the province of Valentia, injected into more than 20,000 persons living cultures of Koch's vibrio. The results published by him did not, however, furnish any real proof of the possibility of conferring immunity against intestinal cholera by means of subcutaneous injections. Later Haffkine[2] modified Ferran's primitive method somewhat, and instead of living vibrios he injected vibrionic cultures killed by heat or by antiseptics. During the cholera epidemic of 1892 and 1893 he tried the inoculation of these killed vibrios into man, with the object of vaccinating against Asiatic

[1] "L'inoculation préventive contre le choléra morbus asiatique" (translated from the Spanish), Paris, 1893.

[2] "Anti-cholera Inoculations in India," *Indian Med. Gaz.*, Calcutta, 1895, No. 1. [Also Report to the Gov. of India, Calcutta, 1895.]

cholera. Later he went to Calcutta in order to try his method on a large scale. He was there enabled to inoculate a great number of persons, and the statistics which he collected appeared to him to be favourable.

But studies on the pathogenesis of Asiatic cholera shook the foundations of Ferran's method. The injections of vibrios, living or killed, were found quite capable of vaccinating animals against vibrionic peritonitis and septicaemia, but they appear to exert no influence whatever against poisoning by the cholera toxin. When it had been learnt how to set up true intestinal cholera in young rabbits Ferran's and other similar methods of vaccination were used in vain to prevent the incidence of this disease, which is very similar to Asiatic cholera of man. An experiment[1] made at the Pasteur Institute in Paris upon two persons vaccinated by Haffkine, showed [504] that they were not protected against the choleriform diarrhoea set up by the ingestion of the cholera vibrios. A third person, who had never been "vaccinated" and who served as "control," after the ingestion of the same cholera culture, behaved exactly as did the other two.

From all these data the conclusion was drawn that in order to prevent intestinal cholera it is necessary to use not cultures of vibrios, living or dead, but antitoxic serums. In fact, the majority of young rabbits vaccinated with these serums and afterwards submitted to infection by the cholera virus through the mouth were found to be vaccinated against intestinal cholera. It has not been possible, as yet, to apply this method to man, hence we are unable to give a decided opinion. Moreover, as the methods based on Ferran's principle have now been abandoned I have not deemed it necessary to devote a special section to anticholera vaccinations. I could not, however, pass it by in silence, since the attempts to vaccinate man against cholera have led to the trial of a similar method against typhoid fever.

Pfeiffer and Kolle[2] were the first to inoculate man with typhoid coccobacilli sterilised by heat. They observed that these injections caused fever, pretty violent pains in the back accompanied by vertigo, shivering and pain at the point of inoculation, without, however, being in any way serious to health. At the same time they found that the blood serum of inoculated persons acquired a very marked protective power (for guinea-pigs injected into the peritoneal cavity with lethal

[1] *Ann. de l'Inst. Pasteur*, Paris, 1893, t. VII, p. 579.

[2] *Deutsche med. Wchnschr.*, Leipzig, 1896, S. 735.

doses of typhoid cultures) quite comparable to the properties discovered by them in the serum of persons who had recovered from typhoid fever. Pfeiffer and Kolle believed that they thus had a proof of the refractory condition of the individuals whom they had submitted to these injections.

These experiments were continued by Wright, Professor of Pathology at Netley, and it is owing to his unwearied efforts that science finds herself in possession of very important evidence on the subject of protective inoculations against typhoid fever in man. According to a verbal communication made to me by Wright, he
[505] has up to the present distributed more than 300,000 doses of his antityphoid vaccine. This vaccine he prepared in the following way[1]. The typhoid coccobacillus is sown in carefully neutralised broth containing 1 °/₀ of peptone. The flasks of culture are kept in the incubator at about 37° C. for two or three weeks, after which their contents are transferred to large flasks in order to be submitted to a temperature of 60° C. This temperature is quite sufficient to kill all the coccobacilli, but for greater surety Wright added to his cultures one-tenth of their volume of a 5 °/₀ solution of carbolic acid or of lysol. The vaccine, thus prepared, is examined as to its toxicity for the guinea-pig by means of subcutaneous injections. Wright injects into man a dose of vaccine which is sufficient to kill 100 grammes of guinea-pig (of the weight of 250 to 300 grammes). This dose often amounts to half a cubic centimetre, but it may have to be increased to 1 c.c. and even 1·5 c.c.

The inoculations are made below the skin of the flank or in the shoulder. They are followed by a rise of temperature which commences as early as two or three hours after the injection. This fever is accompanied by pains in the back, nausea, and want of appetite. There may even be collapse; this led Wright to keep his patient in bed for some time after the vaccinal injection. Besides this reaction, there occurs, at the seat of inoculation, a swelling and redness, accompanied by pain; as a rule all these symptoms have disappeared by the end of 48 hours.

Wright convinced himself that the blood serum of individuals treated by his vaccine, at the end of a certain time acquires the property of agglutinating typhoid coccobacilli in a variable, but usually very marked degree. He even thought that this property

[1] Wright and Leishman, *Brit. Med. Journ.*, London, 1900, Vol. I, p. 122; [Wright, "A short treatise on anti-typhoid inoculation," London, 1904].

might up to a certain point serve as the measure of the immunity acquired against typhoid fever. His own researches, however, showed him that this supposition could not be maintained, and that the agglutinative power, varying greatly in strength, might sometimes be absent where the immunity could not be denied. On the other hand, he clearly showed, especially by the experiments with serum collected at the period which precedes the relapses, that the agglutinative property might be highly developed, in spite of the absence of immunity. Wright then set himself to study the bactericidal property of the serum of individuals who had been injected with his vaccine. [506] He devised a very ingenious method of gaining with a minimum loss of time some idea of the fluctuations of this power of the body fluids to kill the typhoid coccobacillus. In the first place he demonstrated that the bactericidal property is not at all parallel to the agglutinative power, and this has further confirmed him in his opinion that there may be no direct relation between it and acquired immunity. He has found further that the power of the blood serum to destroy the typhoid coccobacillus is very variable in persons vaccinated by his method. After injections of large quantities of these killed bacilli this power may even be diminished for a very long period. On the other hand, medium or small doses of the vaccine first set up a negative stage, during which the bactericidal property is very feeble, and later they bring about an increase of this property, often very marked. Wright does not think that the bactericidal power can serve as the measure of the immunity acquired by the vaccinated individuals, but he hopes that some day a method may be found suitable for the examination of the blood which will give us information as to the degree of immunity conferred by the antityphoid vaccination. For the present the only basis upon which we can form any opinion on this subject is furnished by statistics. Now we know that it is often very difficult to collect data that are sufficiently exact. Hence during the war in South Africa, where one-fifth of the English troops, that is to say about 50,000 persons, were submitted to vaccinations by Wright's method, it is only in certain cases that the statistical information can be utilised. Many of the patients attacked by slight fevers are omitted from the statistics, because from the absence of a precise diagnosis it is not known whether they should come under the category of typhoid patients or not. In other cases the secondary complications divert the attention of the doctors and prevent the registration of a proper diagnosis.

Of the data collected amongst the English troops in South Africa, Wright considers that those which were collected during the siege of Ladysmith were the most exact, on account of the facility with which it was possible to study and register all the cases of typhoid fever under these conditions of complete isolation. Now it has been recognised that, amongst the vaccinated soldiers and officers, there occurred scarcely one-eighth as many cases of typhoid fever as occurred amongst the unvaccinated (1,499 cases in 10,529 unvaccinated, and 35 cases in 1,705 vaccinated). The mortality amongst
[507] the vaccinated was also very much lower. The difference to the credit of the vaccinations should in reality be even greater, for amongst the unvaccinated are counted many persons who having already had an attack of typhoid fever were not submitted to vaccination.

The testimony of the majority of the medical men who followed the results of Wright's method closely is also favourable to the vaccinations. Thus Henry Cayley[1] reports that the staff of a Scotch Hospital of the Red Cross, almost all of whom (57 persons out of 61) had received two vaccinal inoculations, escaped typhoid fever, in spite of the numerous opportunities afforded for the contraction of the disease. This very favourable example is also instructive in that it testifies to the value of two consecutive vaccinations. In many other cases where one has had to be satisfied with a single protective inoculation the results were less brilliant. According to Howard Tooth, who made his observations at Bloemfontein, the vaccinations according to Wright's method must be regarded as very useful.

Outside South Africa this method has been employed on a fairly large number of persons in British India, in Egypt, and in Cyprus. According to the earlier statements from India the incidence amongst the vaccinated persons was one-third that of the unvaccinated. The most recent statistics[2] show still more favourable results. Thus at Meerut the incidence amongst vaccinated persons from Oct. 1899 to Oct. 1900 was one-eleventh that of the unvaccinated (2 cases of typhoid fever in 360 vaccinated, and 11 cases of the same disease in 179 unvaccinated): the mortality (one case amongst the former, six amongst the latter) was less than one-twelfth that of the unvaccinated.

[1] *Brit. Med. Journ.*, London, 1901, Vol. I, p. 84.
[2] *Lancet*, London, 1901, Vol. I, p. 399.

In Egypt and in Cyprus according to the statistics communicated to Dr Wright[1] by Col. Fawcett these vaccinations have given even better results. In 2,669 unvaccinated persons there occurred 68 cases of typhoid fever with 10 deaths, whilst amongst the 720 vaccinated there was only a single case of this disease, this single case succumbing. Here, however, we have to do with a patient who must have received the vaccinal inoculation during the period of incubation, the disease breaking out soon after the vaccination. This would represent in all the cases a morbidity only one-seventeenth as intense amongst the vaccinated.

A few isolated voices only have not pronounced in favour of the [508] antityphoid vaccinations and their opinion is formulated in a very undecided fashion. Amongst the most important of these adversaries, if indeed we may term them such, must be cited Washbourn[2], on account of his experience in microbiology. Attached as a doctor to the Yeomanry Hospital at Deelfontein in South Africa, he witnessed many cases of typhoid fever and was greatly struck by the death of two persons amongst the vaccinated patients. But he himself confesses that it is as yet premature to judge Wright's method, and in support of his sceptical attitude does not offer any other satisfactory observation.

Outside the English colonies vaccinations against typhoid fever have been tried in Russia by Wyssokowitch[3]. He inoculated 235 soldiers of a regiment encamped at Kiew, amongst whom an epidemic of typhoid fever had broken out. The vaccinations were carried out by means of cultures killed with carbolic acid. We are unable to judge of the efficacy of the method because the number of persons vaccinated was too small and the epidemic too limited. It may be noted, however, that amongst these individuals not one took typhoid fever, whilst amongst the unvaccinated three cases of the disease were registered.

The antityphoid vaccinations have as yet only a very short history, and it is, perhaps, premature to express any decided opinion on the matter. We may, however, consider the results already obtained as offering encouragement to continue our experiments. Everything, indeed, tends to a recognition of the utility of vaccinations by means of killed typhoid cultures. The statistics are as a rule good; the

[1] *Lancet*, London, 1901, Vol. I, p. 1272.
[2] *Brit. Med. Journ.*, London, 1900, Vol. I, p. 1456.
[3] *Gaz. clin. de Botkine*, St Pétersb., 1899, p. 1911 (in Russian).

danger from the protective inoculation is *nil* or quite trifling. With the exception of the discomfort of which we have spoken and which is transitory, no untoward result has ever been observed.

To all this must be added the fact that from the point of view of the pathogenesis of typhoid fever, all the probabilities point in favour of the vaccinations. Whilst in Asiatic cholera we have to deal with an intoxication, from the alimentary canal, an intoxication set up by vibrionic products, against which the subcutaneous inoculation of micro-organisms can not be effective, in typhoid fever we have to do with a real infection. The micro-organism, although developed at first in the small intestine, becomes generalised throughout the system. Thanks to improved methods it can always, or almost
[509] always, be found in the blood of the patient, and its constant localisation in the spleen furnishes a real evidence of this. Under these conditions it is quite natural to suppose that everything which is able to prevent the penetration of the typhoid coccobacillus into the blood and the internal organs ought at the same time to contribute to the protection of the individual.

We are fully aware that science has not yet said its final word upon this question. We are coming more and more to the conclusion that it is necessary to make two injections instead of one. It is possible that we may have recourse to certain improvements of the method by combining with it the injections of antityphoid serums as a protective measure. The near future will doubtless bring us the solution of these very important questions.

X. *Vaccinations against human plague.* Plague, which for so long was looked upon as the greatest scourge of humanity, has until recently remained almost unknown from the scientific point of view. But from the moment that it became possible to apply to its study the immense advances realised by microbiology the thick veil which had hidden its nature fell at a single stroke and science found itself in possession of effective means of fighting against it. Amongst these means one of the most important is protective vaccination.

When the last pandemic of plague broke out in Bombay and in the East Indies in general, Haffkine was there engaged in applying his method of vaccination against Asiatic cholera of which we have spoken in the preceding section. Well acquainted with the results of the bacteriological researches made on bubonic plague by Kitasato, and especially by Yersin, he, in 1896, began to study this

disease. After the discovery made by Yersin, Borrel, and Calmette[1], who showed that animals susceptible to human plague could be easily vaccinated against the micro-organism which gives rise to it, Haffkine[2] endeavoured to find a practical method for the vaccination of man. He set up a laboratory at Bombay and, after some preliminary experiments on rabbits, he commenced to inject human beings with pure cultures of the plague coccobacillus. From 1897 up to the present he was able to vaccinate a very large [510] number of individuals, and the results obtained have encouraged him to continue the application of his method. The principle of this method is that which had guided him in the preparation of anticholera vaccines and which is used for the vaccines against typhoid fever. It consists in the employment of pure cultures of the specific organism killed by heat. The cultures are grown in large flasks containing peptonised broth and sown with a small quantity of the plague coccobacilli. A little sterile butter or cocoanut oil is poured on the surface of the fluid. Under these conditions the organism grows abundantly and produces growths which hang down into the fluid, reminding us of the stalactites in a grotto. This mode of development forms one of the most typical characters of the micro-organism of human plague. The culture flasks are kept at a temperature of about 30° C. for five to six weeks, at the end of which period a large number of the bodies of the micro-organisms have fallen to the bottom of the flask, allowing much of their toxic contents to escape. The fatty layer on the surface favours a surface development of the coccobacilli, the number of micro-organisms in a flask being thus greatly increased.

After growing for 35 to 42 days under these conditions the cultures are heated at 65°—70° C. for from one to three hours with the object of killing all the micro-organisms and so rendering their injection innocuous. To make sure of the effectiveness of this heating care is taken to remove a small portion of the fluid and to sow it in a suitable medium. Should this medium remain sterile the vaccine may be used. Into adult men it is injected in a dose of 3 c.c., whilst women, children, and adolescents receive 2—2·5 c.c., into the subcutaneous tissue.

Some hours after the injection of the vaccine the temperature

[1] *Ann. de l'Inst. Pasteur*, Paris, 1895, t. IX, p. 589.

[2] *Brit. Med. Journ.*, London, 1897, Vol. I, p. 1461; *Indian Med. Gaz.*, Calcutta, 1897, Vol. XXXII, p. 201.

rises above normal, reaching 38·5° to 39° C., and sometimes even 40°—40·5° C. This febrile condition lasts from 15 to 48 hours. It is soon accompanied by pain, redness, and swelling at the point of inoculation. These symptoms persist for from three to five days. The *malaise* which follows the vaccinations is sometimes very uncomfortable or even painful, but never serious. Only in exceptional cases is the formation of abscesses observed, and this is due, undoubtedly, to contamination of the vaccines by foreign micro-organisms. The English Commission sent to India to study plague found other micro-organisms than the plague coccobacilli fairly frequently in the
[511] vaccine culture flasks, but, with very rare exceptions, these micro-organisms were found to be innocuous. By rigorously following the rules to be observed in making pure cultures it should not be difficult to avoid this complication.

Haffkine used every effort to induce his patients to be vaccinated a second time, being justly persuaded that two injections are capable of ensuring a more certain and more stable immunity than is a single injection.

From what moment immunity may be considered to be acquired has been a matter for great discussion. From very numerous experiments upon animals of various species, as well as many observations on man, it is now agreed that a period of several days (5—8) from the injection of the vaccine is required before immunity is manifested. It is for this reason that cases of plague which have broken out before this period has elapsed cannot be looked upon as contra-indicating the efficacy of the method.

A large amount of evidence, coming from persons who have made their observations on the spot, is almost unanimous in endorsing the fact that Haffkine's vaccination protects man against plague. It is often difficult to compile exact statistics in surroundings where so many factors contribute to deceive even the careful observer. In spite of this a certain amount of evidence has been collected which may be accepted as affording us fairly satisfactory information. One of the best groups of statistics was that collected at Damaun, a Portuguese possession in India, into which plague was imported from Bombay in 1897, and where a large number of vaccinations were carried out. From the report of Haffkine and Lyons[1], in a population of 8230 persons, rather more than one-fourth (2197) were vaccinated, the greater majority (6033) remaining uninoculated.

[1] "Joint Report on the Epidemic of Plague in Lower Damaun," Bombay, 1897.

Amongst the former only 36 died from plague, which corresponds to 1·6 per cent.; whilst amongst the unvaccinated persons the disease carried off 1482 persons or 24·6 per cent. Vaccination, therefore, according to these statistics, must have brought down the mortality to one-fifteenth. The German Commission[1], two members of which, Koch and Gaffky, went to Damaun to be present at the vaccinations and to observe their efficacy, pronounced in favour of Haffkine's method. The English Commission[2] made reservations and criticised the statistics of Haffkine and Lyons (who amongst others attribute all the cases of deaths that occurred amongst the unvaccinated to [512] plague), but in the end this Commission also recognised the utility of the vaccinations at Damaun.

The data collected with regard to the vaccinations at Undhera, Hubli, and several other places in British India confirm the results obtained at Damaun. The statistics collected at these localities are certainly open to criticism, but the result as a whole is none the less encouraging as regards this method of vaccination. According to the conclusions of the English Commission the "inoculations had a considerable effect in warding off plague attacks from the inoculated....The protection afforded by inoculation seems, however, never to be absolute[3]." We do not, as yet, know the duration of the immunity produced by Haffkine's vaccinations; it cannot be very long to judge from the experiments on animals, but it may last for several weeks, probably even for months.

The vaccinations by killed cultures may be especially useful when it is a question of limiting the extension of an epidemic that is already established. The ease with which the vaccine can be prepared renders it possible to obtain very large quantities of it in a short time, with which it is possible to immunise the entire population of towns or districts. But, as the immunity by this method requires several days for its development and as the injections of micro-organisms, even when killed, may be very injurious during the incubation period of plague or immediately before the infection, it is necessary to limit the vaccinations to persons who are not in intimate contact with the sick, or who are, from the beginning, exposed to infection[4].

[1] *Arb. a. d. K. Gsndhtsamte*, Berlin, 1899, Bd. XVI, S. 331.

[2] "Report of the Indian Plague Commission," London, 1901, Vol. V, Chapter IV.

[3] *Ibid.* Chapter IV, p. 81.

[4] See Calmette, "Rapport sur les vaccinations contre la peste," *Compt. rend. d. X Congr. internat. d'hyg. de Paris*, 1900.

Lustig and Galeotti[1] have described another method of preparing antiplague vaccine which can be utilised where it is of importance to obtain a large quantity of vaccine in a very short time. Instead of allowing the cultures to grow for five or six weeks as required by Haffkine's method, the Italian observers make use of cultures on agar which have grown for two days only. The micro-organisms, removed from the surface of the agar, are treated with a weak solution of potash (0·75 °/₀—1 °/₀) which dissolves the bodies of the coccobacilli.
[513] This phenomenon has sometimes occurred by the end of twenty minutes, but it often requires an hour or more. The contact of the micro-organisms with the alkali must never exceed three hours. The viscous mass thus obtained is then treated with acetic acid, when a precipitate is thrown down. This precipitate, after being washed, is used for the vaccinations. When injected in large quantities into animals, Lustig and Galeotti's product sets up necrosis, but a weak dose is well borne and confers immunity against plague. In man it is sufficient to inject two or three milligrammes of this substance diluted with water. The vaccinal nuclein of the Italian observers has been but little employed for the immunisation of man in India, but it is largely used in this country for the inoculation of horses from which to obtain an antiplague serum.

The serotherapeutics against human plague were inaugurated by the researches of Yersin, Borrel, and Calmette (*l.c.*), who demonstrated that animals susceptible to the plague bacillus can be vaccinated and even cured of experimental plague. The preparation of antiplague serum has since been energetically pursued under Roux's direction at the Pasteur Institute. After several trials, some of which were very encouraging, others, on the contrary, somewhat unfavourable, they succeeded in obtaining a serum which is capable of curing plague after it has broken out and has become grave. As in this treatise we intentionally leave aside everything connected with healing we shall speak only of the antiplague serum as a protective agent.

Whilst vaccinations by killed plague cultures have been practised principally in the East Indies, the immunisation with antiplague serum has been employed in Europe, especially at the time of the epidemics of Oporto in 1899 and of Glasgow in 1900. In all these cases use was made of the serum from the Pasteur Institute, up to the present the most active of all those prepared. It is a serum obtained from

[1] *Deutsche med. Wchnschr.*, Leipzig, 1897, SS. 227, 289.

horses treated for a long period with cultures of the plague bacillus and with the toxin of the same organism. Treatment is begun by injecting plague coccobacilli killed by heat (70° C.). These injections are made into the veins, with the object of avoiding the local lesions which are observed after the subcutaneous introduction of micro-organisms. When the horses have been rendered refractory by this treatment with dead micro-organisms, the next step is to inject (also into the veins) small quantities of living cultures. The doses of these cultures are gradually increased, and end by conferring upon the animal [514] a very strong immunity, which is strengthened by injections of products of cultures passed through a Chamberland filter.

Calmette and Salimbeni[1] injected prophylactically more than 600 persons menaced by plague at Oporto. These comprised the doctors and the staffs of the laboratories of hygiene and of the disinfection services, the firemen who removed the sick persons and the dead, the families of those who were attacked, the members of the French colony, etc. Into each person 5 c.c. of serum was injected below the skin of the abdomen. These vaccinations in some cases caused nettle-rash, eruptions similar to those so often observed after the injection of the other kinds of serums. Of the total number injected two persons contracted plague: the unfortunate Doctor Camera Pestana and his assistant. The former succumbed to the disease, but the second only contracted a very mild form of it. The study of these 600 cases, as well as of experiments on animals, demonstrated that the immunity conferred by the antiplague serum is set up immediately after its injection but is not of long duration. It is probable that it lasts for 8 or 10 days, or at furthest a fortnight only.

Similar results were obtained at Glasgow. Van Ermengem[2], who has published a report on the epidemic in this town, mentions that more than 70 persons in good health were inoculated with the serum; each one received 10 c.c. beneath the skin of the belly. Of these 70 persons one was attacked with a fairly mild plague 8 days after the vaccination, and another, a housekeeper, was attacked, 9 days after the injection, with a congestion of the cervical glands induced by the plague bacillus. Both cases recovered. All the other vaccinated persons, in spite of constant exposure to the plague infection, remained unaffected. Van Ermengem was of opinion that the two

[1] *Ann. de l'Inst. Pasteur*, Paris, 1899, t. XIII, p. 902.
[2] *Bull. Acad. roy. de méd. de Belg.*, Bruxelles, 1900, 27 Octobre.

persons treated with the serum were already infected when they were vaccinated.

The Belgian observer points out, further, the frequency of secondary accidents which were produced in the persons vaccinated at Glasgow. Van Ermengem himself went through the ordeal after being injected with 10 c.c. of serum as a protective measure and this gave occasion to several critics to attack the Pasteur Institute. This is how Van Ermengem himself puts the matter. "The accidents after
[515] the immunising injections...were very numerous, they were observed 33 times in 72 cases. Sometimes they were even fairly serious, to the point of causing great suffering to the patient and of disquieting those around them. We could describe them from thorough knowledge, since we experienced them, but they scarcely differ from those which are observed from time to time after the injection of anti-diphtheria serum, and, like them, they disappear without leaving the least trace" (*l.c.* p. 18).

In spite of these accidents and the necessity of renewing frequently (every ten or fifteen days) the protective injections of serum, their use is quite advisable in certain circumstances. They may render great service on board infected vessels or in lazarettos (as in the case which occurred at Frioul after the arrival at Marseilles of Arab stokers suffering from plague), in docks, warehouses, and stores where contaminated merchandise is found. They should also be employed to vaccinate those coming into immediate contact with plague cases in hospitals and in private houses. In a word, vaccinations by serum, owing to their power of conferring a very rapid immunity, should be practised wherever there is more or less immediate and imminent danger. Under these conditions they are of very great service in localising the disease.

The methods of vaccination against plague that have been employed up to the present may undoubtedly be improved. Calmette and Salimbeni (*l.c.*) have already published the results of experiments on animals undertaken with the object of studying the effect of a combined method of vaccination with antiplague serum and killed cultures of the plague bacillus. But even in their present form the methods used for protecting individuals against this disease deserve to be regarded as conferring great benefits on humanity.

XI. *Vaccinations against tetanus.* Tetanus unlike plague is not a contagious disease, nor is it capable of becoming epidemic.

It constitutes, however, a very formidable disease against which all therapeutic methods have only a very limited effect. This is a further reason for drawing the whole attention of medical and veterinary men to the prevention of tetanus by vaccinal injections. Tetanus is a disease in which the intoxication plays an altogether dominant part. The tetanus bacilli do not develop, at the point where they are introduced into the body, unless favoured by auxiliary conditions, [516] such as the multiplication of other micro-organisms. Even then the organism of tetanus reproduces itself with difficulty, and without becoming generalised throughout the body. The poison which it secretes is however sufficient to produce a very grave intoxication, ending most frequently in death. In certain countries tetanus, as a sequel to various wounds, is very frequently met with in man and in certain domestic animals, such as the horse, donkey, pig, etc.

It is only since the discovery by von Behring and Kitasato of an effective method of immunisation against tetanus that it has been possible to consider the practical application of antitetanus vaccinations. These observers demonstrated that the tetanus poison, when treated with trichloride of iodine, had its toxic action weakened and was transformed into an effective vaccine. Roux and Vaillard found that the addition of Lugol's iodo-iodurated solution to the tetanus poison renders it capable of vaccinating all kinds of susceptible animals. It was shown later, that even with modified active tetanus toxin, we can still obtain good results when care is taken to inject the poison with great circumspection.

But it is not these vaccines obtained from tetanus cultures that have come to be used in practice. The best results are obtained by the use of antitetanus serums. After von Behring and Kitasato's discovery of the power of the serum of animals immunised against tetanus to neutralise the action of the tetanus poison, very numerous experiments were made on the same subject. It has now become possible by treating horses with large quantities of tetanus toxin to obtain specific serums of extraordinary activity Thus several serums are capable of preserving mice against a lethal dose of tetanus poison if we inject into them a quantity of serum equal to the one-thousand-millionth of their weight.

Serums of this strength protect domestic animals against tetanus. We know that many operations on horses, sheep, goats, pigs, and other mammals are very often followed by a tetanus which is usually fatal. Castration, amputation of the tail, the ablation of proud flesh

or tumours, the operation for cryptorchitis or hernias, etc. are often complicated by tetanus. Moreover, tetanus may frequently appear in horses that have received wounds in the foot or in the lower parts of the limbs, "Clous de rue," farrier's punctures, wire-heels, blows, etc.

[517] With the object of remedying this state of things Nocard[1] distributed to veterinarians about 70 litres of antitetanus serum to be employed for protective purposes. The majority of the animals treated (horses, donkeys, mules, bulls, rams, lambs, and pigs) received two injections of serum at an interval of 10—12 days, 20 c.c. for large animals and 6—10 c.c. for sheep and pigs. Of 3088 animals which received the first injection of serum immediately after the operation not a single one contracted tetanus. Of 400 animals which received the first injection at a later period, 1—4 days and more after the accidental wound of which they had been the victims, one horse only, treated five days after the accident (farrier's puncture), was seized with mild tetanus, but it soon recovered. In the same localities where the results of the vaccination were so brilliant, 314 cases of grave and fatal tetanus occurred amongst animals operated upon or injured that were not submitted to the serum treatment.

It may be readily understood with these facts before us why the practice of protective vaccinations of animals against tetanus should have spread so rapidly amongst veterinarians. The demand for antitetanus serum from the Pasteur Institute of Paris for veterinary use increases every year at a great ratio. Thus in 1896 there were sent out only 1511 bottles of 10 c.c. each, in 1898 the number rose to 24,959 bottles, in 1900 it exceeded 43,000.

The efficacy of the antitetanus serum employed as a protective agent can no longer be questioned, but it must not be forgotten that its injection does not render the treatment of the wounds unnecessary. These wounds should receive a rigorous antiseptic cleansing. All foreign bodies should be carefully extracted; otherwise the prolonged presence of tetanus spores might set up a late tetanus after the disappearance of the transient immunity due to the serum.

The protective injections of antitetanus serum into men likely to contract tetanus are also beginning to spread. It often happens that bicyclists, in falling, receive injuries which are contaminated by

[1] *Bull. Acad. de méd.*, Paris, 1895, t. XXXIV, p. 407; *ibid.*, 1897, t. XXXVIII, p. 109; *Compt. rend. XII Congr. Internat. de Méd. à Moscou*, 1897, t. VII, p. 244.

horse-dung or other matters which may contain the spores of tetanus. In these cases, as in many other forms of injury, vaccination with antitetanus serum is indicated. Thus it happens from time to time at the Pasteur Institute that injured persons come and ask for a [518] protective injection of serum. Several medical men and surgeons are now accustomed to vaccinate such of their patients as have had their wounds contaminated by earth or dung. All the cases of this treatment which have come to our knowledge have been followed by very good results.

XII. *Vaccinations against diphtheria.* Antidiphtheria vaccinations have been the subject of much discussion since the discovery of the antidiphtheria serum and its introduction into routine practice. A large number of works were published for and against the application of serum in protective treatment against diphtheria, especially in the early years of its use. Later the controversy has subsided somewhat, and at present very few writers are found who continue to decry antidiphtheria vaccinations.

The antidiphtheria serum was discovered in 1890 by von Behring working in collaboration with Kitasato; these observers demonstrated in laboratory animals its neutralising action upon the diphtheria toxin. A little later von Behring began to apply it in the treatment of diphtheria, but the early results were far from satisfactory, and von Behring soon recognised that it was necessary to obtain much more active serum. Along with Ehrlich of the Institute for Infective Diseases at Berlin he set to work to study this problem. In collaboration with several investigators, among whom I may cite Wernicke, Wassermann, and Kossel, he succeeded in obtaining very encouraging results as regards the antitoxic strength of the serums and their therapeutic action on children attacked by diphtheria.

At this time, also, Roux in Paris began, assisted by Martin and Chaillou, to study the same question. These observers prepared serums which for that period were very active and made a very effective application of them upon more than 300 diphtheria patients.

From the year 1894 the use of serum began to spread in all countries, and it was then that an attempt was made to apply it to the protection of children in good health, but who had been specially exposed to contagion.

It was necessary to have at command large supplies of antidiphtheria serum; this was prepared by injecting into horses

repeated doses of the toxin manufactured by the diphtheria bacillus.
[519] The serums thus obtained were first tested as to their protective, antitoxic, and curative action on guinea-pigs, animals very susceptible to diphtheria. The necessity of finding some means of measuring the strength of the serum soon arose. Von Behring and Wernicke at first standardised it on the basis of the number of grammes of guinea-pig which could be protected by one gramme of serum. Later, von Behring[1] introduced the principle of the "normal serum," that is to say, a serum of which 0·1 c.c., mixed with 10 lethal doses of diphtheria toxin, is capable of preventing every morbid symptom in a guinea-pig weighing 300 to 400 grammes.

Ehrlich[2] perfected this method in the following way: to tubes, each containing 10 lethal doses of a standard toxin, are added different amounts of serum. These mixtures are brought to the same volume of 4 c.c. by the addition of physiological saline solution, and each is immediately injected below the skin of a guinea-pig. If 0·1 c.c. of a serum completely neutralises the 10 lethal doses of toxin, the serum retains its name of normal serum; in the case where 0·05 c.c. is sufficient to bring about the same result the serum is designated double normal serum. When 0·001 c.c. gives the same results, a hundred times normal serum, and so on. A cubic centimetre of normal serum (that is to say a dose capable of neutralising 100 lethal doses of standard toxin) constitutes an "immunising unit" (Immunisirungseinheit (I.E.) of Ehrlich). As it was soon recognised that toxins, even when kept under the best conditions, lose more or less of their toxic power, Ehrlich had to modify his method of standardising serum. He now makes use of a standard antidiphtheria serum, kept in a dry condition, which is much more constant than are the toxins. Solutions of this standard serum are prepared and compared with the serum whose strength has to be determined. Ehrlich has given a detailed description of the method of procedure required to obtain exact results.

At the Pasteur Institute Ehrlich's method has been adopted, supplemented however by another test for the estimation of the strength of antidiphtheria serums, a method allied to von Behring's old method. Various doses of the serum to be examined are injected subcutaneously into guinea-pigs, and 24 hours later these guinea-pigs

[1] *Deutsche med. Wchnschr.*, Leipzig, 1893, S. 390.

[2] Ehrlich, Kossel u. Wassermann, *Deutsche med. Wchnschr.*, Leipzig, 1894, S. 353; *Klin. Jahrb.*, Berlin, 1897, Bd. VI.

receive a quantity of a living culture of diphtheria bacilli which kills [520]
control animals in 30 hours. The protective power of the serum in relation to the weight of the animal is thus determined. For example, a serum which is said to be active at 1/100,000 has the power, in a quantity equal to 1/100,000th of the weight of the inoculated guinea-pig, of preventing a fatal result. It was thought, at first, that the protective power, measured in this way, would be proportional to the antitoxic property determined according to Ehrlich's method. But as the results given by these two methods were often widely different, it was resolved at the Pasteur Institute to examine by both methods all the serums intended for use in practice. This led to the conclusion formulated by Roux[1], in his report communicated to the International Congress of Hygiene, held at Paris in 1900, that a serum possessing a very high protective power (against the living diphtheria bacillus) might be only feebly antitoxic, and *vice versâ*.

This result is explained by the fact that the antidiphtheria serums are very complex fluids, containing several superposed properties of very variable strength. Marx[2], of the Frankfort-on-Main Institute, tried to shake Roux's conclusions, bringing forward his experiments made on guinea-pigs and rabbits injected with antidiphtheria serum into the peritoneal cavity and into the veins. He wished in this way to avoid the introduction of the serum into the subcutaneous tissues, whence the absorption of the antitoxin must take place in a very irregular fashion. In Marx's experiments, thus carried out, the protective power of the serums was always found to run parallel with their antitoxic power, from which he concluded that Roux's view was incorrect. It must not be forgotten, however, that this view was founded on experiments in which the antitoxin had been injected into the subcutaneous tissue before or simultaneously with the toxin or the diphtheria bacillus. Under these conditions the protective power is often found to be altogether disproportionate to the antitoxic power. This fact has been observed so carefully and with such exactness that it is impossible to deny it. Now it is undoubted that the conditions of the experiments upon which Roux relies correspond much more closely with those that are realised in vaccination of man against diphtheria than with the conditions met with in Marx's experiments. In these vaccinations antidiphtheria

[1] *Compt. rend. X Congr. internat. d'hyg. et de démogr.*, Paris, 1900.
[2] *Ztschr. d. Hyg.*, Leipzig, 1901, Bd. XXXVIII, S. 372.

[521] serum is injected below the skin of persons whom it is wished to protect against the action of the diphtheria bacillus.

With the object of bringing about a unification of the methods of estimating serums used in different countries the International Congress of Hygiene, held at Madrid in 1898, appointed a special Commission to settle this problem. But when the Congress met again at Paris in 1900 this Commission had not completed the task allotted to it. The representatives of the various methods had exchanged ideas, but in applying the same method the results obtained in various places and by various observers presented differences too great to allow of any understanding being arrived at. It is evident that we have here a very complicated problem. The serums are tested on living animals in which of course nothing like the constancy of a chemical reaction can be obtained.

Possibly the methods of breeding and the races of the same animals in the different countries may be quite sufficient to explain the divergencies in the results obtained. Whatever may be the reason the unification of serum estimation has not yet been obtained, and it is difficult to anticipate that any better result is to be arrived at.

From all this we may draw the conclusion that the possibility of attaining a too rigorous precision in the standardisation of serum has been exaggerated. Our object must be to obtain results as favourable as possible in the application of the antidiphtheria serums, and for that purpose it is necessary to inject greater quantities than those which may be indicated by any method of estimation. This rule is applied as far as is possible at the Pasteur Institute.

As regards vaccination against diphtheria of persons who are in good health but are especially exposed to infection, the question must be accepted as settled in the affirmative.

From the commencement of our attempt to cure diphtheria by means of a specific serum, the necessity was seen of protecting children who were in contact with the sick persons against this disease. Small quantities of serum were injected into such children for protective purposes. The first results communicated in 1894 by Roux to the Congress at Budapest being very encouraging, an attempt was made to give the greatest possible extension to the system of vaccination by antidiphtheria serum. In the following year, 1895, fairly

[522] numerous statistics had been collected, and Torday[1] at Budapest,

[1] *Deutsche med. Wchnschr.*, Leipzig, 1895, S. 408.

Kurth[1] at Bremen, and Rubens[2] at Gelsenkirchen were able to publish a number of favourable statistics. Soon afterwards, however, a fatal case occurred in the family of a well-known Berlin doctor, Langerhans[3], an accident that started a violent controversy and stirred up an active campaign against serum. Langerhans's son, a boy aged 2 years, in good health, was inoculated with a small dose (1·2 c.c. of this serum) and succumbed about a quarter of an hour afterwards with symptoms of suffocation. The post-mortem examination made by Strassman[4] showed the cause of death to be suffocation in consequence of the aspiration of food into the respiratory passages during the act of vomiting. An examination of the serum used by Langerhans did not reveal any toxic action on animals or any contamination by micro-organisms. All to no purpose, the serum was held answerable for the death of the child, and an attempt was made to demonstrate at almost any cost that its use in human practice was extremely dangerous. Gottstein[5] joined in chorus with the over-excited opinion and published a denunciation of vaccinations by antidiphtheria serum. He collected from the literature of both hemispheres four cases, in all, in which death had occurred some time after the injection of this serum into children not suffering from diphtheria. A perusal of the description of these cases is sufficient to convince one that the death could in no sense be attributed to the serum, and that it could be explained much more easily by the fatal action of the streptococcus, the cause of the non-diphtheritic affections of the children that died.

The ineptitude of this denunciation must have done much to calm public opinion, and in September of the same year, 1896, C. Fränkel[6], in a report presented to the German Association of Public Hygiene, was able to give a review of the state of the question of vaccination against diphtheria, summing up in favour of the use of the specific serum. "Taking into consideration the data collected," he remarks, "it is scarcely possible to doubt the value of immunisation by serum, [523] so that we may say positively that we are now treading a path which will lead us to great and important results." This very favourable

[1] *Deutsche med. Wchnschr.*, Leipzig, 1895, SS. 426, 443, 464.
[2] *Deutsche med. Wchnschr.*, Leipzig, 1895, S. 758.
[3] *Berl. klin. Wchnschr.*, 1896, S. 602.
[4] *Berl. klin. Wchnschr.*, 1896, S. 516.
[5] *Therap. Monatsh.*, Berlin, 1896, S. 269.
[6] *Deutsche Vrtljschr. f. öff. Gsndhtspflg.*, Brnschwg., 1897, Bd. XXIX, Heft 1.

opinion was due in great measure to the vaccinations carried out in the wards of Heubner's Clinic at Berlin[1]. At first, injection of the antidiphtheria serum as a protective into patients who were found in the immediate vicinity of the children attacked with diphtheria (contacts) was deemed to be sufficient: but in consequence of the results obtained by this method it was decided (starting from January, 1896) to inject all children who came into the hospital. During the first period there still occurred a few cases of diphtheria contracted in hospital, but from the moment systematic and general vaccinations were introduced not a single new case occurred.

The immune condition of the vaccinated children is maintained for three to four weeks. After this lapse of time some of them contracted diphtheria. But it was sufficient to introduce revaccination at the end of this period to prevent the outbreak of any further case of diphtheria in Heubner's wards. Results quite as favourable and as convincing were obtained in the department for children attacked by scarlet fever.

The amount of serum injected varied, but it was usually given in doses of 1 c.c. containing from 200 to 250 I.E. (immunising units of Ehrlich). The serum was always found to be innocuous except in certain cases where it set up erythemata of greater or less extension. In 460 injections 20 cases of these exanthemata were produced, that is to say 4·34 °/₀. The frequency of these complications was not proportional to the amount of serum injected. According to the figures communicated by Löhr the largest doses of the serum employed did not produce exanthemata more frequently than did the smaller quantities. Thus 117 injections of 1 c.c. only were followed in five cases by these erythemata, which corresponds to 4·27 per cent. The hope of diminishing the frequency of the exanthemata by diminishing the amount of serum injected was therefore not realised. This fact lends support to the conclusion above formulated as to the exaggeration of the importance of the measurement of serum. If it could be established that small quantities of serum rich in antitoxin caused cutaneous eruptions less frequently than did stronger doses
[524] there would certainly be a great advantage in using serums containing a very large number of immunising units for vaccination. Perhaps serums having a great antimicrobial power but of comparatively low antitoxic potency might even render great service in protective

[1] See the report by Löhr in *Jahrb. f. Kinderh.*, Leipzig, 1896, Bd. XLIII, S. 67.

treatment. Future researches undertaken in this direction alone can give us information on this subject.

In 1896 the vaccinations in Heubner's wards were discontinued, but the reappearance of diphtheria in 1897[1] rendered their recommencement necessary. 500 children were vaccinated each with 200 immunising units. Following this no case of diphtheria broke out. The eruptions were rare and slight.

The increasing extension of the use of antidiphtheria serum for the cure of the disease after it has broken out has led to a greater development in its use as a preventive measure. Thus, in the countries where diphtheria is endemic, vaccinations by serum are now practised very extensively. In Russia, which is one of the great hotbeds of this disease, vaccinations by antidiphtheria serum are frequently practised.

At the Congress of Russian doctors at Kasan in 1896, Vissotsky communicated the result of 2,185 vaccinations which gave a morbidity of 1·3 °/₀, a morbidity that must be regarded as very low indeed. A well-known Russian physician for children's diseases, Rauchfuss[2], who cites these figures, has collected several other facts concerning the prophylactic injections of antidiphtheria serum followed by good results. In the government of Woronetz, according to the statements of Ouspensky[3], out of 738 vaccinated persons diphtheria occurred in 2·2 per cent., which again may be considered a favourable result, especially if we take into account the great extension of diphtheria in this country. In Podolia, out of 537 children vaccinated in 1895, only four cases of diphtheria occurred, a morbidity of 0·74 °/₀. In the government of Kherson, one of the great centres of diphtheria in southern Russia, the results appear to be less favourable: out of 543 children which received a protective inoculation, 21 contracted the disease (or 4·6 per cent.), of which five died. If we study these statistics more closely[4] it will be seen that these results are far from being unfavourable. The protective inoculations [525] were made only once and with somewhat small doses, nevertheless many of the cases of diphtheria broke out only at a late period, sometimes more than nine months after the injections had been made. Now, it is proved that these injections, although very efficacious,

[1] See Slawyk., *Deutsche med. Wchnschr.*, Leipzig, 1898, S. 35.

[2] "Les progrès dans l'application du sérum antidiphthérique," St Pétersbourg, 1898, p. 105 (in Russian).

[3] *Vrach*, St Pétersbourg, 1900, p. 1178 (in Russian).

[4] *Chron. méd. d. gouvern. de Kherson*, 1896, No. 5, p. 160 (in Russian).

produce their action for a very short time only, for a few weeks at most. Of the five fatal cases, four did not occur until 2, $4\frac{1}{2}$, 6, and $9\frac{1}{2}$ months respectively after the protective inoculation. It is impossible to look upon these statistics as affording proof of the inefficacy of the serum. The fifth case is the only one that occurred within a short time (15 days) of the injection, and in this instance only 150 immunising units had been injected.

A detailed study of the other examples of antidiphtheria inoculations in the government of Kherson leaves a very favourable impression. Out of 90 children inoculated by Wecker[1] in the district of Elisabetgrad not a single one contracted diphtheria, which is the more remarkable as at the time of the inoculations there existed in the same families 14 cases of diphtheria; the chances of contamination were thus great.

Recently, on the occasion of the outbreak of a great epidemic in Paris, the question of vaccinations by serum was again raised and earnestly discussed at the Paris Hospitals Medical Society and at the Society for the Study of Children's Diseases. Voisin and Guinon[2] communicated the history of an epidemic amongst the staff at the Salpêtrière Hospital in the wards of idiot children, "against which protective serum treatment was remarkably effective and absolutely innocuous." The serum was injected, in the case of children more than 10 years of age, in 10 c.c. doses, and into the rest in 6 c.c. doses. This measure brought about first an abatement and then cessation of the epidemic. The immunity after a single injection lasted from two to three weeks, and the few cases of diphtheria which broke out amongst the infected children were distinguished by their great mildness. Erythemata and other post-injection complications were insignificant, so that the protective use of the serum was fully justified. Only a small minority of the medical men who took part in the discussion spoke against the antidiphtheria vaccinations; once, indeed, a reference was made to the case of Langerhans's child, although its death was certainly not due to the serum. It is true that in families where it is possible to keep the children under careful observation and to intervene at the appearance of the first
[526] symptoms of diphtheria, the preventive injections may be dispensed with, but in practice these favourable conditions are rarely realised,

[1] *Chron. méd. d. gouvern. de Kherson*, 1896, No. 19, p. 743.
[2] *Bull. et mém. Soc. méd. des Hôp. de Paris*, 1901, p. 585.

and the prophylactic serum treatment is then of great service in preventing the outbreak of the disease.

Netter[1] communicated to the Society of Pediatrics a summary of 32,484 observations on the prophylactic injection of antidiphtheria serum. Of this number 192 cases were noted in which the diphtheria broke out in spite of the injections, corresponding to 0·6 per cent. of those treated. These figures, however, included all cases of the disease which occurred up to thirty days after the injection. Now, the immunity is often less durable than this, and it may disappear more or less completely twenty days and sometimes even fifteen days after vaccination.

Netter himself made great use of antidiphtheria vaccination. It was his custom to propose to the parents either a protective inoculation at once or a systematic precautionary bacteriological examination of the throats of the children not yet attacked. He regards the first method as preferable. According to the latest statistics which he was kind enough to communicate to me, of 152 children (in 50 families), 91 of whom received protective inoculations, not one contracted diphtheria: whilst in 239 other families where the children had not been inoculated there were 52 cases of diphtheria, with 10 deaths. Many practitioners in Paris have now pronounced themselves in favour of protective injections of the serum, and the Society of Pediatrics, at its meeting on 11th June, 1901, concluded the discussion of this question by proposing the following resolution: "The Society of Pediatrics, affirming that protective inoculations present no serious danger and confer a very considerable amount of immunity for some weeks, recommend their use when children are gathered together in numbers, and in families where a scientific supervision cannot be maintained."

The large amount of evidence collected on this question leaves no doubt as to the real efficacy of vaccinations by antidiphtheria serum.

The summary of the results obtained by vaccination in the 12 diseases of man and of animals I have just placed before my readers cannot pretend to serve as a detailed guide to prophylactic practice. My object has been merely to concentrate into one chapter [527] the principal data upon which this very important question rests, to bear witness to the progress which has already been realised, and at the same time to show that the scientific study of immunity is in

[1] *Bull. Soc. d. Pédiatr. de Paris*, 1901, mai et juin.

very intimate relation with its practical application. It is evident that the road is far from traversed to its terminus, for there are many infective diseases in which vaccinations cannot be employed, but it is none the less certain that the path which has led to so many important and useful results should still be followed in studying problems which up to the present we have been unable to solve.

CHAPTER XVI

HISTORICAL SKETCH OF OUR KNOWLEDGE ON IMMUNITY

Methods used by savage races for vaccination against snake venom and against bovine pleuropneumonia.—Variolisation and vaccination against small-pox.—Discovery of the attenuation of viruses and of vaccinations with attenuated micro-organisms.—Theory of the exhaustion of the medium as a cause of acquired immunity.—Theory of substances which prevent the multiplication of micro-organisms in the refractory body.—Local theory of immunity.—Theory of the adaptation of the cells of the immunised organism.

Observations on the presence of micro-organisms in the white corpuscles.—History of phagocytosis and of the theory of phagocytes.—Numerous attacks upon this theory.—Theory of the bactericidal property of the body fluids.—Theory of the antitoxic power of the body fluids.—Extracellular destruction of micro-organisms.—Analogy between bacteriolysis and haemolysis.—Theory of side-chains.

Progress of the theory of phagocytes.—Attempts to reconcile it with the humoral theory.—Present phase of the question of immunity.

As protection against disease is one of the most important amongst those questions which are engrossing the attention of humanity, it is natural that very great attention should have been devoted to it from the most remote times. We see primitive races, the ordinary layman, medical men, legislators and even the most subtle thinkers devoting their energies to the solution of the problem of immunity against poisoning and against infections. Historical science will never reveal to us the earliest sources of our knowledge on this question, so remote are their origins. The wide distribution of several methods for protecting man and cattle against certain diseases clearly proves that the origin of this practice dates from a very early period.

The frequency of venomous snakes in many countries has inspired a dread of these reptiles, and this must have led to the search for

some method of fighting against the poisoning after the patient had been bitten. Thus, we find that many primitive races make use of various methods of immunising the body against the action of
[529] venom. The Portuguese colonel, Serpa Pinto[1], in a letter addressed to d'Abbadie, describes the method by which he was vaccinated by the Vatuas, natives of the east coast of Africa. These savages extract the poison of snakes and prepare from it, by the addition of vegetable substances, a very brown glutinous paste which they introduce into incisions made in the skin. This operation is very painful and is followed by a swelling which lasts for a whole week. The Vatuas assert that this method confers a sure immunity against the venom. Serpa Pinto was never bitten by a snake, but, a short time after he had been vaccinated, he was stung, in the Seychelles Islands, by a scorpion without experiencing any ill effects. This experience confirms the assertion of the Vatuas, because it has been shown that the vaccine against snake venom is also efficacious against the bite of scorpions. The fact that after being stung by another scorpion ten years later Serpa Pinto was so ill that for eight days he believed that he was going to die or at least to lose an arm, shows that he did not enjoy natural immunity, and the innocuousness of the previous bite must therefore be attributed to a vaccination the effect of which had disappeared at the end of ten years.

Another vaccinal method used by primitive races is that against the pleuropneumonia of the Bovidae. De Rochebrune[2] points out that the Moors and the Pouls of Senegambia have "a custom whose origin is lost in the obscurity of antiquity" which consists in the inoculation into their herds of cattle of the virus of the epizootic pleuropneumonia. "The point of a knife of primitive form, or of a dagger, is plunged into the lung of an animal that has died from the disease and an incision, sufficient to allow the virus to penetrate below the skin of the healthy animal, is made into the supranasal region. Experience has demonstrated the success of this protective operation."

In Europe, the vaccinations of cattle with the virus of pleuropneumonia have certainly been known for more than a century, for, in a pamphlet published at Berne in 1773[3], mention is made of the "inoculation" of Bovidae as a means of preventing the disease in

[1] *Compt. rend. Acad. d. sc.*, Paris, 1896, t. CXXII, p. 441.
[2] *Compt. rend. Acad. d. sc.*, Paris, 1885, t. C, p. 659.
[3] This pamphlet has been reprinted in the *Rec. de méd. vét.*, Paris, 1886, p. 624.

England and in Holland, a disease against which it has been recognised that remedies are powerless.

The inoculation of the variolous virus into the healthy human [530] subject, which comes into the same category as the inoculation of the pleuropneumonic virus into healthy bovine animals, is also a widely extended and very ancient method. The Chinese[1] assert that they have known from the commencement of the 11th century the method of immunising against small-pox. Amongst them, as amongst the Siamese, the matter from the variolous scab is introduced into the nostrils. In Persia variolisation is practised by surgeons and by the staffs of bathing establishments, who introduce the powdered scabs into scratches in the skin. The Ashantis inoculate the variolous virus into seven places on the arms and legs. According to the account of Timoni, a Greek physician practising in Constantinople in the first half of the 18th century, the Circassians and Georgians, intent upon preserving the beauty of their daughters, make punctures at various points in the skin, with needles charged with variolous virus. Everybody is acquainted with the fact that it was from Constantinople that Lady Mary Wortley Montague at the same period (1721), imported into Europe "the Greek method," which consisted in the inoculation of the contents of small-pox pustules with the object of producing a benign small-pox and of protecting the vaccinated person from severe and dangerous small-pox. This practice was widespread in Europe during the second half of the 18th century, but as it was not unattended by serious drawbacks an attempt was made to avoid them by the employment of all kinds of medicaments. As these, however, were found to be entirely ineffective, the need was felt of replacing variolisation by some more benign method.

It is asserted[2] that in Baluchistan the custom of having cows suffering from cow-pox milked by children who had wounds on their hands has been widespread from time immemorial. This practice conferred upon these children an immunity against small-pox. It cannot be denied that the idea of being able to vaccinate with cow-pox was common knowledge amongst breeders and dairymen in several countries in Europe, especially in England, France, and Germany. It is stated that Edward Jenner learnt from the country people of his native county of Gloucestershire that contact with cow-pox

[1] Barthels, "Die Medicin der Naturvölker," Leipzig, 1893, S. 128; Pagel, "Einführung in die Geschichte der Medicin," Berlin, 1898, S. 313.

[2] Häser, "Lehrbuch der Geschichte der Medicin," 3te Aufl., Jena, 1881, Bd. II S. 1075.

protected against small-pox. Being a man of great understanding and culture, he set himself to verify this opinion experimentally. Having
[531] demonstrated by a great number of experiments that the inoculation of variolous virus into persons vaccinated by cow-pox had no ill result, he became the great propagandist of the new method. He worked at this subject for 20 years but only decided to publish his results (in 1798) after he had completely satisfied himself of the great utility of vaccination with the virus of cow-pox. At first Jenner's discovery met with great opposition, but his method was soon verified in France and several other countries and it was not long before it was generally practised.

When Pasteur set himself to study the infective diseases in their relation to micro-organisms the idea of profiting by the discovery of these pathogenic organisms and of drawing from them a weapon against infections soon arose in his mind. He studied Jenner's work in order to extract from it any indications capable of putting him into the right path. He induced his collaborators to carry out several series of experiments with the object of immunising the animal organism against infective micro-organisms. During this laborious and original work chance[1] helped in the accomplishment of his task. When, at the conclusion of the holidays in the autumn of 1879, Pasteur and his collaborators Chamberland and Roux wished to resume their experiments on fowl cholera, they found to their great surprise that the micro-organisms of this disease, usually so fatal, had become innocuous. Fowls, that received doses of cultures much more than sufficient to cause death, did not experience any ill effect. Prepared by his previous knowledge and by the continual direction of his thoughts to the prevention of contagious diseases, Pasteur divined at once the great bearing of this check in his inoculations with old cultures, and immediately began to make precise experiments as to the vaccinating power of these micro-organisms which had become innocuous. These researches led him to the discovery of two great principles: that of the attenuation of viruses, and that of the vaccinating property of attenuated micro-organisms. Various memoirs by Pasteur[2] established these laws in a very exact manner; moreover he gave all the information necessary to allow of the

[1] See Vallery-Radot, "La Vie de Pasteur," Paris, 1900, p. 427.

[2] *Compt. rend. Acad. d. sc.*, Paris, 1880, t. xc, pp. 939, 952, 1030; t. xci, pp. 571, 673.

principal results being controlled and verified. In France, this great discovery was at once accepted by various investigators, though others found occasion to manifest their scepticism. Abroad this [532] discovery met with very lively opposition and this from the highest authorities, who would not recognise the possibility either of attenuating the virus or of conferring immunity upon animals. The anthrax bacillus can be grown for a very long time on culture media, the potato, for example, without losing its pathogenic power in the slightest degree. Therefore, it was said, this attenuation of virus can have no actual existence. White rats that have resisted one or more inoculations of the anthrax bacillus may die from a later inoculation of the same micro-organism. Therefore there is no acquired immunity, etc. The principles laid down by Pasteur are from every point of view of such prime importance, that very numerous experiments were carried out at once for the purpose of verifying their exactness and the contest was not a long one. In the course of a few years it was universally recognised that the attenuation of viruses, and also the vaccination by attenuated micro-organisms, were realities which henceforth cannot be denied and which must pass into the domain of truths definitely acquired. An attempt was then made to extend these fresh victories to the other infective diseases. Pasteur, Chamberland, and Roux applied themselves to devising a method of vaccinating animals against anthrax and against rabic virus; Pasteur and Thuillier extended their researches on this subject to swine erysipelas. From several other quarters the search for vaccines was instituted. Toussaint made various attempts, at times crowned with success, to immunise animals against anthrax by means of heated anthrax blood. Arloing, Cornevin, and Thomas succeeded in vaccinating the Bovidae against symptomatic anthrax. Loeffler was the first in Germany to demonstrate that rabbits which had recovered from the disease set up by the bacillus of mouse septicaemia acquired an immunity against the attacks of this organism. It is not necessary to cite further examples, so numerous have they become and so unanimously confirmatory.

After the first steps had been taken along this new path Pasteur and his collaborators began to apply the knowledge they had gained to the preparation of vaccines capable of giving practical results. The two anti-anthrax vaccines and the two vaccines against swine erysipelas were the fruit of these attempts. Here, again, numerous objections were raised against these discoveries. Sheep which had

[533] received enormous quantities of the bacillus may die from anthrax in spite of the two Pasteurian vaccines and from that it was wished to conclude that these vaccines should not be employed in practice to protect sheep against the anthrax fever. The results of experiments made on a large scale in various parts of the globe have demonstrated the inadequacy of these objections and these questions are now regarded as definitely settled.

So large a number of investigations, in response to the most urgent and immediate needs, was not favourable to minute researches on the mechanism of this immunity which had been revealed in so marvellous a fashion. In spite of this, Pasteur applied himself to the solution of this problem so far as this was possible under the conditions in which he carried on his investigations. He thought that acquired immunity was the result of the impossibility of the growth of a pathogenic micro-organism in a medium in which it had previously been cultivated. When the micro-organism of fowl cholera sets up in certain individuals a disease which though grave is not fatal, or when the attenuated micro-organism produces a simple, transient discomfort, it lives in both cases in the fluids and tissues of the animal. This existence is possible in consequence of the absorption of certain nutrient substances. Once these substances are consumed they are not easily renewed, and in consequence the vaccinated organism becomes incapable of nourishing the special micro-organism a second or a third time. To support this brilliant hypothesis by precise facts Pasteur made experiments on the conditions met with in the development of the micro-organism of fowl cholera *in vitro*. He filtered a broth culture of this micro-organism after it had grown luxuriantly for several days, and into the fluid, which had now become clear and transparent, he sowed afresh the same micro-organism. No growth took place and the fluid remained quite clear. This absence of development might be explained either by the presence in the fluid of some excremental substance thrown off during the first culture or by the absence of some substance indispensable for the nutrition of the micro-organism. Pasteur excluded the first hypothesis by an experiment which demonstrated that it is sufficient to add to the filtered fluid a small quantity of fresh nutritive substances to enable the micro-organism again to develop abundantly. It is therefore to the absence of some element essential to the existence of the micro-organism that we must attribute the immunity enjoyed by animals which have been vaccinated or which

have undergone spontaneous cure. This is how Pasteur[1] expressed himself on this point: "the muscle which has been much affected [534] has, even after healing and repair, become in some way incapable of supporting the growth of the micro-organism, as if the latter, by a previous culture, had eliminated from the muscle some principle that life does not bring back and whose absence prevents the development of the small organism. There is no doubt that this explanation, to which the plainest facts at the moment lead us, will become general and applicable to all the virulent diseases."

This explanation appeared to be a reasonable one to several observers, amongst whom I may cite Chauveau[2], the distinguished author of important works on viruses. "In all probability this seductive theory," says Chauveau, "based on one of the most interesting of those clear and decisive experiments for which Pasteur is famous, applies to the majority of cases of immunity acquired by protective inoculation." But Chauveau thinks that it does not explain natural immunity, especially that of the Algerian sheep, against anthrax, an example that he had studied on several occasions. When he inoculated into these animals large quantities of anthrax bacilli, not going beyond certain limits, the sheep resisted perfectly; but injections of enormous doses were nearly always capable of overcoming this natural immunity of the Algerian sheep and of inducing in them a fatal anthrax. Chauveau thinks that this fact is best explained by the presence of an inhibitory substance in the blood plasma, whose action becomes exhausted when distributed over a very large number of bacilli. This opinion was not, however, shared by Pasteur[3], who raises the objection that natural immunity can really be produced and maintained without the presence of this inhibitory substance from the fact that fowls, which exhibit such marked resistance against anthrax, readily contract the disease when the temperature of their bodies is lowered. Under these conditions it is unimaginable that an inhibitory substance has disappeared under the influence of cold.

The controversy existent from the birth of theories on immunity shows us that from the very commencement the problem was found to be a very complex one, and that to attack it in a satisfactory way we must as far as possible multiply and deepen our study of the phenomena which accompany the resistance of the animal against

[1] *Compt. rend. Acad. d. sc.*, Paris, 1880, t. XC, p. 247.
[2] *Compt. rend. Acad. d. sc.*, Paris, 1880, t. XC, p. 1526.
[3] *Compt. rend. Acad. d. sc.*, Paris, 1880, t. XCI, p. 536.

pathogenic micro-organisms. Thus, Chauveau[1] was not long before
[535] he undertook experiments having for their object the determination of the fate of anthrax bacilli when injected into the blood vessels of Algerian sheep. He found that these organisms disappeared from the blood at the end of a few hours, but they were then to be found accumulated in the lung, spleen, and certain other viscera. In these positions the bacilli become incapable of reproducing themselves and in refractory individuals soon disappear, being opposed by the inhibitory substances of the blood plasma.

The two theories just sketched have this point in common, that they both attribute the natural or acquired immunity to humoral and purely passive properties. According to one theory it is the impoverishment of the fluids of the animals which prevents the development of the pathogenic organism, whilst according to the other it is the presence of some bacterial poison which brings about the same result. To give experimental support to his theory Pasteur brought forward his attempts at sowing micro-organisms in culture media exhausted by a previous development of the same organism, eliminating, so to say, the active influence of the animal organism. It is true that, in order to explain natural immunity, it was necessary to ascribe a rôle to the "constitution" and to the "vital resistance," interpreting this, as Naegeli had already done, in the sense of a competition for the oxygen and the nutritive substances between the parasites and the cells of the body.

Adopting this point of view, Hans Buchner[2], a pupil of Naegeli, attempted to gain a more precise idea of the conditions under which acquired immunity against infective diseases is set up. He developed his theory in various publications; this theory consists, briefly, in the property of the animal organism to reinforce the local resistance of the organs by means of an inflammatory reaction. The starting-point of this local theory is the thesis that each pathogenic micro-organism can only manifest its pathogenic action when it enters the particular organ in which it is capable of living and maintaining itself. Thus, the pneumonococcus can live in the lungs only, the cholera vibrio in the intestines only, and so on. Every time that a pathogenic micro-organism becomes localised in its special organ, an inflammatory action is set up which results in the reinforcement of the living

[1] *Compt. rend. Acad. d. sc.*, Paris, 1880, t. XCI, p. 680.

[2] "Die Naegeli'sche Theorie d. Infectionskrankheiten," Leipzig, 1877; "Eine neue Theorie über Erziel. v. Immunität," München, 1883.

elements of the organ in question. Inflammation, therefore, is regarded by Buchner as a salutary reaction, which acts, not directly [536] on the exciting morbific cause, but through the mediation of the specific cells of the organs. This theory of immunity led Buchner to propose arsenical treatment as a remedy against microbial disease, because arsenic is, of all drugs, the one capable of setting up the greatest inflammatory reaction.

Another German observer, Grawitz[1], proposes a theory of acquired immunity, according to which a first attack of an infective disease sets up "the adaptation of the cells to the power of energetic assimilation of the fungi." This reinforced adaptation is transmitted to the descendants of the cells which have acquired it, and for that reason the immunity may persist for months, and even years. Grawitz attempted to base his views on experiments on the acquired immunity against the fungus of the lily of the valley, but Loeffler[2] soon demonstrated that this thesis could not be maintained, and that the immunity assumed by Grawitz did not, in reality, exist.

It will be seen that all the theories summarised above are marked by their vague character and want of precision; this is not at all astonishing when we take into consideration the very imperfect knowledge of the phenomena of immunity. It is evident that if we wish to gain a satisfactory idea of the mechanism of the resistance of the animal body against pathogenic micro-organisms, we must inform ourselves as to the modifications which take place in the organs and tissues at the time of the acquisition of the immunity, and also find out what becomes of the micro-organisms in a refractory animal.

We have seen that Chauveau demonstrated that anthrax bacilli when injected into the vessels of Algerian sheep disappear, but he was unable to say anything as to the way in which this disappearance was brought about in nature. Buchner accepted the reinforced resistance of inflamed organs without being able to describe the phenomena which manifest themselves during the inflammation of tissues invaded by the pathogenic micro-organisms.

Independently of these theoretical and rather speculative views on immunity, there has been an addition to our scientific assets of fairly exact data on the relation of certain pathogenic organisms to the organs and tissues of susceptible or refractory animals. When, as

[1] *Virchow's Archiv*, 1881, Bd. LXXXIV, S. 87.
[2] *Mitth. a. d. k. Gsndhtsamte*, Berlin, 1881, Bd. I, S. 134.

[537] a result of the labours of Davaine and Obermeyer, the attention of pathologists, especially of those working at pathological histology, was drawn to the part played by micro-organisms in infective diseases, a diligent search was instituted for these organisms in sections of the organs of persons who had died from various diseases. Masses of cocci especially were found in the organs of individuals who had died from diphtheria, puerperal fever, and various forms of pyaemia. In the course of these investigations attention was drawn fairly frequently to the presence of micro-organisms inside the white corpuscles of pus and of other morbid products. Amongst the first to make this observation I may cite Hayem[1] in France, and Birch-Hirschfeld[2], Klebs, Rindfleisch, von Recklinghausen, and Waldeyer in Germany. Klebs[3] speaks of the presence of micro-organisms in infected wounds, in the interior of contractile white corpuscles, and attributes to these cells the principal rôle in the transport of these parasites in the lymphatic tissue. Waldeyer[4] cites a case of puerperal fever in which the corpuscles of the peritoneal pus were filled with bacteria. Similar observations were by no means rare; and they led to a general conclusion that micro-organisms meet with such favourable conditions inside the leucocytes that they would contribute to their dissemination through the body. This opinion had become so general that when Koch[5], in frogs inoculated with anthrax bacilli, made the discovery of round cells containing large numbers of these micro-organisms he did not hesitate to conclude that the bacilli found a favourable medium in the substance of these elements. Now the frog, under ordinary conditions, is refractory to anthrax.

As early as 1874, however, Panum[6] had given expression to the view, in a vague fashion it is true, that leucocytes might assist in the destruction of micro-organisms. In his memoir on putrefactive poisons we find a note wherein occurs the following reflection:

[538] "For the solution of the question as to how and in what situations the ordinary bacteria of putrefaction disappear, an interesting communication made by Birch-Hirschfeld seems to me to furnish an

[1] *Compt. rend. Soc. de biol.*, Paris, 1870, p. 115; *Gaz. hebd. de méd.*, Paris, 1871, p. 291.

[2] Abstract in *Schmidt's Jahrb.*, Leipzig, 1872, Bd. CLX, S. 97.

[3] "Beiträge zur pathologische Anatomie der Schusswunden," Leipzig, 1872.

[4] *Archiv f. Gynaek.*, Berlin, 1872, Bd. III, S. 293.

[5] Cohn's *Beitr. z. Biol. d. Pflanzen*, Breslau, 1876, Bd. II, S. 300.

[6] *Virchow's Archiv*, 1874, Bd. LX, S. 347.

indication. According to this observer the micrococci, introduced into the circulation, are deposited in the lymphatic glands and in the spleen, after having, for the most part, entered into the blood corpuscles. That the ordinary bacilli of putrefaction really die in the body is proved, not only by the circumstance that they remain inactive after the acute paroxysm of putrid intoxication has been happily surmounted, but also by the important observations made by Eberth on the innocuousness of the inoculation of ordinary bacteria into the cornea." These lines contain the indication that the corpuscles of the blood (in this case undoubtedly leucocytes) ingest the bacteria introduced in the blood current and destroy them.

Some years later, in 1877, Grawitz[1], in connection with his researches on the parasite of the lily of the valley, made the remark that the fungi, when introduced into the blood of mammals, are seized by the white corpuscles and thus "withdrawn from contact with the assimilable fluid." Gaule[2] who, as we know, sought to demonstrate that the *Drepanidium* of the frog's blood is nothing but the fragments of cell nuclei transformed into 'Würmchen,' has described the structure of these organisms in the amoeboid cells of the spleen. "I happened on one occasion," he writes, "to observe an amoebocyte of the spleen of the frog which in a short time ingested three 'Würmchen,' and then went away briskly without leaving any trace of where it had been. Following its movements I was able at the first to make out within the contents of the amoebocyte the refractile body of the 'Würmchen.' But this body became paler, and half-an-hour later it had been completely assimilated." Undoubtedly these "Würmchen" were nothing but parasites (*Drepanidium*), and have no connection with the cell nuclei of frogs. Their ingestion, followed by destruction, was, therefore, a defensive act on the part of the body manifested by the amoeboid cells of the splenic pulp.

In the same year, 1881, in which this observation by Gaule was published, Roser[3], assistant in surgery at Marburg, published a small pamphlet on the lower animals. In this pamphlet the possibility of growing certain unicellular organisms in urine and milk and the [539] adaptation of these organisms to saline solutions received special mention. At the end of one of his paragraphs Roser expresses his

[1] *Virchow's Archiv*, 1877, Bd. LXX, S. 546; 1881, Bd. LXXXIV, S. 87.

[2] *Archiv f. Physiol.*, Leipzig, 1881, S. 308, Taf. v.

[3] "Beiträge zur Biologie niederster Organismen," Marburg, 1881.

views on immunity, although this subject was not discussed at all in his pamphlet. He expresses himself thus: "The immunity of animals and plants in complete health depends in my opinion: (1) on the relative quantity of salt contained in their fluids, and (2) on the property of their contractile cells of ingesting the enemy which enters the animal body" (p. 18). As these statements have been put forth without receiving any further development, in the midst of all kinds of other speculations, it is not astonishing that the words I have just quoted, as well as Roser's pamphlet itself, should not have attracted the attention of either zoologists or medical men. In the reviews for these two sciences (Schmidt's *Jahrbücher* and the *Zoologischer Jahresbericht* of the Zoological Station at Naples) it is not even mentioned. It appears that not only did other biologists and medical men attach no importance to Roser's speculations, but that the author himself did not claim any great value for them. I draw this conclusion from the fact that five years after his first pamphlet he published a second on inflammation and healing[1] in which he does not apply his theory of immunity to explain these two phenomena. This new work is of an even more speculative character than was the first, and instead of attempting to show any relation between the anti-infective part played by the leucocytes and their migration during inflammation, Roser insists on the fundamental independence of this phenomenon of healing. For him the inflammation, accompanied by diapedesis, must not be looked upon as a healthy reaction of the body, but as a manifestation of disease. The heat which is observed under these conditions must be attributed in part at least to the production of heat by infective micro-organisms. I must confess that Roser's two pamphlets were unknown to me for many years, and it was Hueppe who drew my attention to them by his mention of them in the fourth edition of his work on bacteriological methods[2] which appeared in 1889. I had then, independently of the Marburg surgeon and by a totally different path, arrived at my conclusions as to the part played by the amoeboid cells. At the commencement of my researches on healing and immunity the
[540] passages cited above from the publications of Panum, Gaule, and Grawitz were also unknown to me. Having long studied the problem of the germinal layers in the animal series, I sought to gain some idea of their origin and significance. The part played by the

[1] Roser, "Ueber Entzündung und Heilung," Leipzig, 1886.

[2] "Methoden der Bacterienforschung," 4te Aufl., Wiesbaden, 1889, S. 10.

ectoderm and the entoderm appeared quite clear, and the former might quite reasonably be regarded as the cutaneous investment of primitive multicellular animals, whilst the latter might be regarded as their organ of digestion. The discovery of intracellular digestion in many of the lower animals led me to regard this phenomenon as characteristic of those ancestral animals from which might be derived all the known types of the animal kingdom (excepting, of course, the Protozoa). The origin and the part played by the mesoderm appeared the most obscure. Thus, certain embryologists supposed that this layer corresponded to the reproductive organs of primitive animals: others regarded it as the prototype of the organs of locomotion. My embryological and physiological studies on sponges led me to the conclusion that the mesoderm must function in the hypothetically primitive animals as a mass of digestive cells, in all points similar to those of the entoderm. This hypothesis necessarily attracted my attention to the power of seizing foreign corpuscles possessed by the mesodermic cells. This fact has long been recognised. It was known that the white corpuscles of the Vertebrata often contained various kinds of cells, especially red and white blood corpuscles. It was known, also, that the amoeboid cells were capable of ingesting granules of coloured substances. When making an injection of indigo into the vessels of *Thetys*, Haeckel[1] in 1858 was surprised to find the blue granules inside the amoeboid blood corpuscles of this beautiful gasteropod mollusk. This fact has since been confirmed by many observers, and the capacity of the amoeboid cells to take up foreign bodies became recognised as a general phenomenon. Nevertheless this phenomenon was not regarded as being analogous to digestion. Thus Haeckel[2] himself, in his researches on the calcareous sponges, advocated the view that the foreign bodies penetrated into the interior of the viscous protoplasm in a purely passive fashion.

Observations that I made on sponges and on certain pelagic [541]
animals, transparent and of simple organisation, convinced me that the presence of foreign corpuscles in the amoeboid cells of the mesoderm must be attributed to an active ingestion by these cells which, in every respect, might be compared to the phenomena of intracellular digestion in the epithelial cells of the digestive canal of many of the lower animals. In order to demonstrate this fact

[1] "Die Radiolarien," Berlin, 1862.
[2] "Die Kalkschwämme," Berlin, 1872.

clearly it was necessary to bring forward exact experimental proof. I set myself, therefore, during my stay at Messina in 1882 and 1883, to study the rôle of the amoeboid cells of the mesoderm from the point of view of intracellular digestion. I found it an easy matter to demonstrate that these elements seized foreign bodies of very varied nature by means of their living processes, and that certain of these bodies underwent a true digestion within the amoeboid cells. My principal thesis, that is to say the idea of the intimate relations between the entoderm and the mesoderm, was thus fully confirmed.

Pondering over these results, which were quite new at the time, the idea suggested itself to me that the digestive function, so profoundly rooted in the mesodermic elements, must play a part in many of the vital phenomena of animals. Starting from this standpoint, I succeeded in demonstrating that, during the very complicated metamorphoses of Echinoderms, such as the *Synaptae*, the amoeboid cells of the mesoderm fulfil a function in the atrophy of numerous larval organs. I have never prosecuted any medical studies ; but some time before my departure for Messina I listened to the reading of Cohnheim's treatise on General Pathology, and I was struck by his description of the facts and of his theory of inflammation. The former, especially his description of the diapedesis of the white corpuscles through the vessel wall, seemed to be of momentous interest. His theory, on the other hand, appeared to be extremely vague and nebulous. It occurred to me that a comparative study of inflammation in lower animals of simple organisation would certainly throw light on the very complex pathological phenomena in the Vertebrata, even in the frog which had served as the starting-point for Cohnheim's remarkable experiments.

Since, in the atrophy of the larval organs of the *Synaptae*, the essential rôle is accomplished by the amoeboid cells of the mesoderm which accumulate and unite into masses, the richness of inflammatory exudations in white corpuscles may perhaps signify that these cor-
[542] puscles have a very important function to fulfil. This reflection led me to make the following experiment: to wound and introduce spines beneath the skin of very transparent marine animals ; if my hypothesis should be well founded this should bring about an accumulation of amoeboid cells at the injured spot. I selected for this purpose the large Bipinnaria larvae of star-fish, so abundant at Messina, and inserted prickles of the rose into their bodies. Very shortly these

prickles were found to be surrounded by a mass of amoeboid cells such as we see in human exudation as the result of the introduction of a spine or other foreign body. The whole process took place under my eyes in a transparent animal possessing neither blood nor other vessels, nor a nervous system. The first point was settled. The inflammatory exudation must be considered as a reaction against all kinds of lesions, the exudation being a more primitive and more ancient phenomenon in inflammation than are the functions of the nervous system or of the vessels.

I know quite well that, at the period when I made my researches (1882), pathologists regarded inflammation as the consequence, if not always, at least in the majority of cases, of the penetration of micro-organisms. From this followed the conclusion that the diapedesis and accumulation of white corpuscles in inflammatory diseases must be regarded as modes of defence of the organism against micro-organisms, the leucocytes in this struggle devouring and destroying the parasites. According to this hypothesis the significance of inflammation at once became simple and clear. With the object of verifying my hypothesis I began to make experiments on the lower animals, so abundant in the Straits of Messina, and to make myself acquainted with the results that had been obtained in general pathology and in pathological histology. A perusal of Ziegler's treatise on Pathological Anatomy made it clear to me that in these branches of medical science there had long been accumulated a great number of observations fitted to facilitate the acceptation of the new hypothesis on inflammation and healing. Numerous and well-established facts on the absorption of extravasated blood, on the fate of the coloured corpuscles in the body, on the presence of micro-organisms inside leucocytes, etc., confirmed me in my view.

When I had got together certain information and a number of facts in support of my hypothesis I communicated the results to my lamented friend, Kleinenberg, at that time Professor in the University of Messina. Both medical man and zoologist, he was well qualified [543]
to offer a judgment upon the matter ; this judgment was favourable. Sometime later I had the great pleasure of meeting the celebrated Professor Virchow at Messina. I imparted to him my ideas and he was kind enough to come with me to examine my preparations of Bipinnaria larvae and other lower animals in which I had set up the phenomena of inflammation without the assistance of nervous or vascular systems. This eminent observer greatly encouraged me to

continue my investigations. When I explained to him my view that the inflammatory reaction on the part of the amoeboid cells could only be understood by accepting the hypothesis that the white corpuscles gave chase to the micro-organisms and destroyed them, Virchow replied that in pathology just the opposite was invariably taught. The general opinion was that micro-organisms were certainly found inside the leucocytes and that they made use of these cells as a means of transport and of dissemination through the body.

During my stay at Messina my researches were limited to the lower animals, but later I began to study inflammation and the phenomena of infection in the Vertebrata. It was not until eight months after I had commenced my researches in this direction that I decided to publish my results. I first set them forth in an address given at Odessa before the Congress of Naturalists and Medical Men in 1883. Later, they were published in a special article inserted in Claus's *Arbeiten* at Vienna[1], and in a small work which appeared in the *Biologisches Centralblatt*[2]. I sought especially to develop the idea that the intracellular digestion of unicellular organisms and of many Invertebrata had been hereditarily transmitted to the higher animals and retained in them by the amoeboid cells of mesodermic origin. These cells, being capable of ingesting and digesting all kinds of histological elements, may apply the same power to the destruction of micro-organisms. In order to support this conclusion I introduced various kinds of bacteria into the bodies of some of the lower animals and I demonstrated that they were ingested and destroyed by the amoeboid cells. It was evident, however, that this proof was not sufficient. I then set myself to study the diseases of small Invertebrata sufficiently transparent to be observed directly under the microscope. The *Daphniae*, those small crustacea so numerous and so frequent in fresh water, furnished me with a favour-
[544] able medium in which to study a real struggle which takes place between their leucocytes and the spores of a vegetable parasite belonging to the group of the Blastomycetes. In many cases the amoeboid cells guarantee the integrity of the animal by devouring a large number of these spores and transforming them into an inert detritus. In other cases, on the contrary, the fungi get the upper hand in the struggle ; they succeed in germinating and in overcoming the resistance of the leucocytes by reproducing themselves rapidly

[1] *Arb. a. d. zool. Inst. d. Univ. Wien*, 1883, Bd. v, S. 141.
[2] *Biol. Centralbl.*, Erlangen, 1883, Bd. III, S. 560.

and by killing these cells with their poisons. The history of this disease and of this struggle was published in *Virchow's Archiv*[1].

Some time afterwards I published in the same journal my work on the anthrax bacillus[2], in which I attempted to demonstrate that in the Vertebrata also the invasion of pathogenic micro-organisms sets up a desperate struggle between them and the amoeboid cells.

In these four works I made use of the term "phagocytes" to designate the amoeboid cells capable of seizing and digesting the micro-organisms and other formed elements. To the theory based on this property of the defensive cells I gave the name of "theory of phagocytes."

I thought, as already mentioned above, that the observations on absorption and leucocytes, which had been accumulating for years in pathological histology, had sufficiently paved the way for a favourable reception to the idea that the amoeboid cells are defensive elements of the body capable of guaranteeing to it immunity and cure. In this I was mistaken. It was precisely the specialists in this branch of science who from the first manifested the most lively opposition to this theory.

However, in the Presidential Address delivered before the 66th meeting of the British Association held at Liverpool in 1896, Lord Lister said[3]: "If ever there was a romantic chapter in pathology, it has surely been that of the story of phagocytosis." These words encourage me to put before the reader the essential features of this story.

My first two memoirs published in 1883 did not in any way attract the attention of the medical public. These investigations had a character that was too zoological to be noticed by patholo-
gists. But the two following publications, in which I treated of the [545]
Daphnia disease and especially of bacterial anthrax, immediately roused severe criticism. Baumgarten[4], the well-known pathologist, opened the battle by the publication of a review of my researches on phagocytosis. He attempted to sap the basis of my theory, and not contented with *à priori* arguments, he set his pupils to make a series of researches on the fate of micro-organisms in the refractory

[1] *Virchow's Archiv*, 1884, Bd. XCVI, S. 177.

[2] *Virchow's Archiv*, 1884, Bd. XCVII, S. 502.

[3] *Rep. Brit. Ass. Adv. Sci.*, London, 1896, p. 26; *Rev. Scient.*, Paris, 17 Octobre, 1896, p. 493.

[4] *Berl. klin. Wchnschr.*, 1884.

animal. These researches resulted in several theses for the doctor's degree which sought to demolish every point of the theory of phagocytosis.

Later, Baumgarten[1] published a long and above all admirably written analytical article entitled: "Zur Kritik der Metschnikoff"-schen Phagocytentheorie," in which, with much talent and wit, he attempted to demolish the bases and conclusions of the phagocytic theory.

Baumgarten regards the precise observations which I had been accumulating for some years as incorrect and refuted by the observations and experiments of his pupils. The arguments that I give to justify my theory are, according to the same critic, contrary to logic and to truth. If the phagocytes are really elements destined to guarantee the integrity of the animal organism how is it, asks Baumgarten, that just at the moment of greatest danger, when the blood and the tissues are invaded by the micro-organisms, the leucocytes are conspicuous by their absence? The answer that there is no predestination in the phagocytosis, and that the danger is the greater the more feeble the phagocytic reaction—a fact which is in perfect harmony with the law of causes and with the principles of the evolution of species according to Darwin's theory—did not satisfy my critic. He says: "If the interpretation which Metschnikoff gives of the activity of the leucocytes appears to be rather the product of a rich imagination than the result of the objective observation of the seeker, it matters little that his account of the development of the leucocyte in what he wishes to see in it should be in conformity with the principles of the theory of evolution" (p. 4).

I was able by numerous researches[2] to refute point by point the
[546] objections based on the work of Baumgarten's pupils, but that did not prevent him from persisting in his negation. Only, commencing by writing long articles, he contented himself, later, with denying the theory of phagocytosis in small annual notes, appearing in his reviews of works on bacteriology, which were unsupported either by argument or by any facts mentioned in his abstracts.

Baumgarten's example was followed by many other pathologists. Ziegler, the well-known author of a text-book on pathological anatomy that has certainly had a wider circulation than any other

[1] *Ztschr. f. klin. Med.*, Berlin, 1888, Bd. xv, S. 1.

[2] *Virchow's Archiv*, 1888, Bd. cxiv, S. 465; *Ann. de l'Inst. Pasteur*, Paris, 1890, t. iv, p. 35.

work, vigorously attacked the theory of phagocytosis. As it was precisely from this treatise that I had acquired my knowledge of the large number of facts that had accumulated in pathological literature on the part played by leucocytes in resorption, I was persuaded that Ziegler, who had collected these statements, would be one of the first to recognise the importance of phagocytosis in inflammation, healing, and immunity. But this distinguished pathologist, in several of his publications[1], expressed himself very vigorously against the phagocytic theory. The intervention of these cells, according to him, must be purely accidental and their rôle in the defence of the body against the micro-organisms very insignificant. The better to demonstrate this thesis he caused his pupils to undertake investigations on several infective diseases, and these young observers all arrived at the same result, that phagocytosis has nothing to do with the struggle of the animal against the anthrax bacillus or against the bacillus of symptomatic anthrax. It is the less necessary to enter into these details now because I have, in the preceding chapters, given sufficient proofs of the incorrectness of the objections advanced by Ziegler's school. It has been demonstrated most conclusively (by Lubarsch's researches, as well as by many other works) that in anthrax in man phagocytosis, denied by one of Ziegler's pupils, is most marked. It is likewise well known from the researches of Ruffer, Leclainche and Vallée, as well as from my own observations, that in symptomatic anthrax, in which the phagocytic reaction is denied by another of Ziegler's pupils, it is a very important and highly developed feature.

The opposition emanating from another eminent pathologist, Weigert[2], particularly impressed me, because this investigator is known not only to be an observer of great accuracy but to possess a mind of great imagination and generalising power. In several [547] papers he put forward his utmost ingenuity to demolish the phagocytic theory root and branch. He would recognise neither the importance of phagocytosis in healing and immunity, nor the defensive function of the giant cells. Weigert, however, contented himself with formulating theoretical objections, and no works directed specially against the doctrine of phagocytosis have issued from his laboratory. It must be stated, however, that although there has been such oppo-

[1] "Lehrb. d. pathol. Anat.," Jena, 3te Aufl.; *Beitr. z. path. Anat.*, Jena, 1889, Bd. v, S. 419.

[2] *Fortschr. d. Med.*, Berlin, 1887, Bd. v, S. 732; *Ibid.*, 1888, Bd. vi, SS. 83, 809.

sition on the part of certain of our most eminent pathologists, others amongst them have, from the beginning, expressed themselves in more favourable terms. Thus, Virchow[1], in an introductory article in the 101st volume of his *Archiv*, continued his friendly attitude with regard to the works on phagocytic defence and spoke of them as opening up a new field of research. Ribbert[2], in a series of publications, maintained the importance of the phagocytes in the resistance offered by the animal to the aggression of micro-organisms, and pointed out, especially in connection with the diseases set up by the staphylococci, the frequency of the ingestion of these parasites by the leucocytes. He insists specially on a modification of the phagocytic reaction, which consists in the accumulation of white corpuscles around the centre of microbial infection. In these cases, without the occurrence of any real ingestion of the micro-organisms into the substance of the phagocytes, these organisms may have their morbific manifestation hindered by the assemblage of the white corpuscles. It is needless to insist that this act, which I referred to in my first work in 1883, constitutes the prelude to a true phagocytosis and is closely bound up with this defensive phenomenon. Another pathologist, Hess[3], supports the theory of phagocytosis by confirmatory researches of great value.

The pathologists who were adversaries of the phagocytic theory combined their efforts to demolish it, without troubling themselves to replace it by any other theory of defence on the part of the body which might more easily be made to accord with their principles and their statements. Baumgarten certainly tried to prove that micro-organisms perish in cases where immunity is produced or healing occurs, not as the result of the phagocytic reaction or of any other manifestation on the part of the menaced animal, but simply "of themselves" (von selbst), that is to say, they have simply accomplished the normal cycle of their existence and die a natural
[548] death, this bringing about healing and immunity. As may be readily understood he was unable to bring forward the slightest evidence of the correctness of this hypothesis, which, I believe, has never been accepted by anyone, nor even been defended by its author. In this respect the attacks directed against the theory of phagocytosis by bacteriologists have been of a very different character. Not content

[1] *Virchow's Archiv*, 1885, Bd. CI, S. 12.
[2] *Deutsche med. Wchnschr.*, Leipzig, 1890, S. 690.
[3] *Virchow's Archiv*, 1887, Bd. CIX, S. 365.

with overturning this hypothesis, these observers have sought to build upon its ruins new theories capable of offering a better explanation of the phenomena of immunity. I must here confess at the outset that these attacks have been much more important than those coming from the pathologists and pathological anatomists, and have led to discoveries of the greatest value.

One of Fodor's experiments[1], one not altogether new, served as the point of departure for much work and for a large series of objections directed against the phagocytic theory. The Hungarian investigator found that the defibrinated blood of the rabbit was capable of destroying *in vitro* a great number of anthrax bacilli. From this it was concluded that the fluids of the living body possessed a bactericidal power sufficient to explain the immunity against infective micro-organisms. The destruction of the anthrax bacillus by defibrinated blood was confirmed by a young American investigator of great talent, Nuttall[2], who carried out an important work on this subject in the laboratory and under the direction of Flügge at Breslau. He was able to follow step by step, by the observation of anthrax bacilli on the warm stage, their degeneration under the action of the defibrinated blood. This destruction of the bacilli took place outside the phagocytes. The same phenomenon could be shown by the method of gelatine plate cultures. The bacilli, subjected to the influence of the defibrinated blood of rabbits and other vertebrates, usually died or were markedly injured. The blood when heated to 55° C. completely lost its bactericidal power.

These observations, perfectly exact in every detail, gave Flügge[3] and his assistant Bitter[4] the opportunity to criticise vigorously the theory of phagocytosis. The cells were said to be incapable of [549] ingesting living micro-organisms; these latter must be previously destroyed by the bactericidal action of the body fluids, and it was only their dead bodies which were devoured by the phagocytes.

Flügge based his criticism upon considerations of a general character and upon observations made mainly by Nuttall. "There is no necessary point of analogy," says the learned Breslau hygienist, "between the ingestion of food and the struggle against infective

[1] *Deutsche med. Wchnschr.*, Leipzig, 1886, S. 617; *Arch. f. Hyg.*, München u. Leipzig, 1886, Bd. IV, S. 129.

[2] *Ztschr. f. Hyg.*, Leipzig, 1888, Bd. IV, S. 353.

[3] *Ztschr. f. Hyg.*, Leipzig, 1888, Bd. IV, S. 223.

[4] *Ztschr. f. Hyg.*, Leipzig, 1888, Bd. IV, S. 318.

micro-organisms, nor between nutritive substances and living micro-organisms" (p. 225). "From Nuttall's results it must evidently be accepted as possible that the phagocytes can ingest dead bacteria only and that they have not the power of ridding the body of the living infective agents" (p. 226). The following passage is especially significant. "When we examine, with an open mind, a series of preparations which show the relations between the phagocytes and the bacteria in various infective diseases, the phagocytes sometimes present themselves as the victims of the bacteria, which continue their triumphal march; sometimes they produce the impression of tombstones lying in large numbers behind the line of battle and after the end of the struggle. On the other hand, they in no way force themselves upon our notice as instruments of slaughter which the attacked organism makes use of to defend itself" (p. 227).

These arguments have been regarded by many investigators in all countries as perfectly sufficient to overthrow the phagocytic theory. The bactericidal power of the body fluids became the rallying cry of a great number of works always directed to the same object: to replace the rôle of phagocytosis by that of a bactericidal power of the body fluids. It is quite unnecessary to weary the reader with a list of the very numerous publications that have appeared on this subject in every European language. But it is not possible to pass over in silence the work of some of the principal partisans of the humoral theory of immunity.

The first place amongst these works certainly belongs to von Behring's memoir[1] on the natural immunity of white rats against anthrax. As already stated in Chapter VI of this work, von Behring discovered the very remarkable power possessed by the rat's blood
[550] of destroying anthrax bacilli with very great rapidity. This investigator did not hesitate to conclude therefrom that this bactericidal property of the blood must, in the rat, bring about a great resistance against anthrax. We should have in this case, then, an example in which the immunity did not depend in any way upon phagocytosis, but would be bound up entirely in a purely humoral property.

With the object of deciding whether the bactericidal property of the blood is really the general and essential cause of natural or acquired immunity, von Behring, in collaboration with Nissen[2], carried out a long series of experiments, the results of which, however, did

[1] *Centralbl. f. klin. Med.*, Bonn, 1888, No. 38.
[2] *Ztschr. f. Hyg.*, Leipzig, 1890, Bd. VIII, S. 412.

not confirm their expectations. They found that in animals well vaccinated against certain bacteria (notably Gamaleia's vibrio or *V. metschnikovi*), the blood plasma undoubtedly acquires a high specific bactericidal power, but at the same time they satisfied themselves that the blood, even of well immunised animals, was generally incapable of killing the micro-organisms. The bactericidal property, then, according to their researches, presented itself not as a general character but as one of limited importance. These facts even led von Behring to abandon the theory of the bactericidal power of the body fluids as an explanation of immunity.

This theory found many warm partisans, especially at Munich. Emmerich had already announced at the International Congress of Hygiene, held at Vienna in 1887, that in the blood of rabbits vaccinated against the bacillus of swine erysipelas an antiseptic substance of remarkable activity is produced. To this, exclusively, in this instance, and not to the phagocytes, he attributed the acquired immunity. Later, Emmerich[1] in an investigation carried out in collaboration with di Mattei developed this view. We may refrain from giving any account of the contents of their memoir as well as from criticising their conclusions, as this has already been done in Chapter IX. Let us content ourselves with stating that our own experiments, as well as those made later by Mesnil, have demonstrated the inaccuracy of Emmerich's statements.

Another Munich bacteriologist, H. Buchner, at first expressed himself[2] very favourably on the theory of phagocytosis. He regarded it as more capable of explaining most of the phenomena of immunity than was his own older local theory. But little by little he declared himself in formal opposition to the cellular theory of immunity and went over to the camp of his sometime adver- [551]
saries. He adopted[3] the humoral theory of the bactericidal action of the body fluids, upon which subject he carried out several important investigations. He was able without difficulty to confirm Nuttall's discovery of the disappearance of the microbicidal power when the defibrinated blood was heated to 55° C., and he added to this fundamental fact many others of great value. He demonstrated the part played by the salts in the exercise of this bactericidal power, and laid great stress on the fact that this power depends on the

[1] *Fortschr. d. Med.*, Berlin, 1887, Bd. v, S. 653.
[2] *München. med. Wchnschr.*, 1887.
[3] *Centralbl. f. Bakteriol. u. Parasitenk.*, Jena, 1891, Bd. x, S. 727.

presence of a special substance of albuminoid nature, to which he gave the name of *alexin*. Buchner[1] combatted with success the idea that I had expressed, according to which the bactericidal power of the body fluids is reduced in great part to a plasmolytic action of the blood serum upon certain micro-organisms. It cannot be denied that my hypothesis is only very partially applicable, and that the larger share in the bactericidal action of the body fluids belongs to the alexins. Buchner also made the study of this action more easy by the demonstration that the red blood corpuscles of a foreign species undergo, under the action of the blood and of the serums, a globulicidal action comparable to that which occurs in the case of micro-organisms.

Whilst Flügge, von Behring and many others of the old partisans of the bactericidal theory of the body fluids abandoned it more or less completely as an explanation of immunity, Buchner remained faithful to it and tried, aided by the collaboration of his pupils, as far as possible to defend it.

In France this humoral theory was adopted chiefly by Bouchard[2] and his pupils, amongst whom I must cite more particularly Charrin and Roger. They sought to confirm it by personal researches, the greater part of which were carried out upon the bacillus of blue pus. These investigators studied it especially in relation to acquired immunity. A comparison of the mode of development of the pyocyanic bacillus in the serum of susceptible animals and of vaccinated animals of the same species, convinced them of the great importance of the action of the body fluids. In cases where these fluids were found to be incapable of killing the micro-organisms they exerted over them an injurious influence, either by attenuating their virulence,
[552] or by producing more or less important modifications in their forms and functions. The essential cause of natural or acquired immunity was always attributed by Bouchard's school to the property of the body fluids. The phagocytes were said to intervene only secondarily, either to carry off the dead bodies of the micro-organisms, or to ingest the bacteria, rendered inoffensive by the humoral action.

The humoral theory of immunity, with some slight modifications, spread very generally into every country, and many investigators accepted it without reserve. But certain observers ventured to run counter to the general current and raised objections of principle

[1] *Centralbl. f. Bakteriol. u. Parasitenk.*, Jena, 1890, Bd. VIII, S. 65.

[2] "Les microbes pathogènes," Paris, 1892.

against the theory of the bactericidal power of the fluids of the body. After the principal facts established by the partisans of this theory had been confirmed, it was asked whether the phenomena of the destruction of micro-organisms observed *in vitro* are really equivalent to those produced in the refractory animal. A glance at the data brought together with so much zeal was sufficient to demonstrate that this parallelism does not exist. The blood of animals susceptible to certain micro-organisms was found to be bactericidal for these organisms, whilst that of refractory animals was incapable of destroying them. It is useless to cite examples, so numerous are they. On the other hand, the bactericidal power of the body fluids, so marked for certain pathogenic organisms such as the anthrax bacillus and especially the cholera vibrio and the typhoid coccobacillus, is insignificant or *nil* as regards many bacteria against which refractory animals are not wanting.

All these facts throw doubt on the predominating part played in immunity by the bactericidal power of the body fluids. Lubarsch[1] attacked the humoral theory, showing by a great number of experiments that animals whose fluids are very bactericidal *in vitro* are very susceptible to a much smaller quantity of bacteria of the same species introduced into the body. Thus, the defibrinated blood and the blood serum of rabbits destroy a large number of bacteria in a very short time, whilst the rabbits themselves contract fatal anthrax after the introduction of a small number of these micro-organisms into the blood vessels. This contradiction cannot be [553] explained except by the profound changes which the blood must undergo outside the body. Facts of the same nature have been shown for the anthrax of rats by Hankin, Roux, and ourselves, as described in Chapter VI.

The International Congress of Medicine, assembled at Berlin in 1890, was the first occasion on which I spoke publicly of the new theories of immunity. In the addresses given at the general meetings, leaders of medical science in several countries summed up their opinion on this question. Koch[2], in his memorable report, declared that the new acquisitions had destroyed the basis of the theory of phagocytes, and that consequently it must give place to the humoral theory of immunity. Bouchard took up a more conciliatory position, but, according to him, the bactericidal power of

[1] *Centralbl. f. Bakteriol. u. Parasitenk.*, Jena, 1889, Bd. VI, SS. 481, 529.

[2] "Ueber bacteriologische Forschung," Berlin, 1890.

the fluids of the body was the primary and essential cause of immunity. The phagocytes only intervened later, in order to finish the work begun without their assistance. Lord Lister expressed himself[1], on the other hand, much more favourably on the subject of the theory of phagocytosis. This observer, who is not only a great surgeon, but is perhaps even more remarkable for his great powers of generalisation, has paid special attention to the problem of immunity. With the object of clearing up this very complicated and at the same time important question, Lord Lister seized the occasion of the meeting of the International Congress of Hygiene in London in 1891, to bring about an exchange of views between the partisans of the various theories of immunity. Under his presidency he devoted an entire sitting of the Section of Bacteriology to the discussion of this question. Buchner presented a report[2] drawn up exclusively from the point of view of the humoral theory and devoted to the demonstration of the slight importance of phagocytosis, and also to the preponderant part played by the alexins dissolved in the body fluids and circulating in the plasma of the blood. He attempted to harmonise the facts on the bactericidal power of serums observed *in vitro* with the special conditions to be met with in the animal body. He specially insisted on the point that, in the blood and the organs, the alexins cannot act with the same rapidity that they can [554] in test-tubes containing serum. In this way he recognised that between the bactericidal action *in vitro* and that in the body of the animal, there exists a marked difference, but he would not consent to attribute it in the latter case to the intervention of the phagocytes.

Roux[3] also made a report on immunity at the same sederunt, speaking very distinctly in favour of the cellular theory. A chemist by inclination, he was sympathetic at first to the humoral theories of immunity. Working with Pasteur, and side by side with him, Roux, from the beginning of the new era of medical science, had made numerous experiments on the part played by the body fluids in immunity. But as the results were not sufficiently precise and demonstrative they were soon abandoned. The attachment of Roux, however, to the humoral theories was manifested in his work, carried out in part with Chamberland[4], on the subject of vaccination by

[1] "The present position of antiseptic surgery," Berlin, 1890.
[2] *München. med. Wchnschr.*, 1891, SS. 551, 574.
[3] *Ann. de l'Inst. Pasteur*, Paris, 1891, t. v, p. 517.
[4] *Ann. de l'Inst. Pasteur*, Paris, 1887, t. I, p. 561.

means of microbial products. Later, having obtained a deeper knowledge of various facts concerning natural and acquired immunity, he rallied to the cellular conception and developed it in his report presented to the above Congress in London. Several microbiologists took part in the discussion, and I myself[1] was able to communicate certain facts concerning the immunity of guinea-pigs, acquired as the result of vaccination against Gamaleia's vibrio. I chose this example because it presented, according to von Behring and Nissen, the clearest case of a bactericidal property developed during the course of immunisation. I was able to furnish the proof that, in the vaccinated animal, the micro-organism in question, in spite of the great bactericidal power of the blood serum *in vitro*, remains alive in the animal body for a long time, and that its destruction is effected by the phagocytes, which ingested it alive. In this example I showed that the leucocytes of the exudation, that have ingested vibrios, may still furnish cultures of this organism if they are taken from the body and transferred in hanging drop to the incubator.

The fact that, even in the case which appeared most to favour the humoral conception of acquired immunity, phagocytes play the principal part, must to many members of the Congress have appeared sufficiently significant. Indeed, several observers who were present at the debates, received the impression that the phagocytic theory had not been overturned by its adversaries. At this period the [555] question of the importance of antitoxins from the point of view of immunity had scarcely been raised. The great discovery made by von Behring and Kitasato was already accepted by everyone; but there was no ground for attributing to it any general importance. In fact, though proved for tetanus and diphtheria, and extended by Ehrlich's beautiful experiments to the vegetable toxins (ricin, abrin, and robin), the antitoxic property of the fluids of the body presented itself rather as a special than as a general phenomenon. It is in this sense that Roux had assigned to it its place in the chapter of immunity. The two diseases, against which antitoxic serums had been discovered, are certainly distinguished from the great majority of infections by the localisation of the micro-organisms and the abundant secretion of their toxins.

It was only after the London Congress that this question came prominently forward. Von Behring thought that the antitoxic power of the body fluids is generally distributed in all cases of acquired

[1] *Ann. de l'Inst. Pasteur*, Paris, 1891, t. v, pp. 465, 534.

immunity, and that micro-organisms, introduced into the animal possessing this power, become incapable of any pathogenic manifestation. Certain facts, brought together in Bouchard's laboratory, tell against the hypothesis I have just mentioned. With the object of throwing light on this question I began, immediately after the close of the Congress, to study the acquired immunity of rabbits against the micro-organism of the pneumo-enteritis of pigs. I was able to demonstrate[1] that in this case the resistance of the animal against the micro-organisms does not depend on the acquisition of any antitoxic property by the body fluids; such a property is completely absent. At the same time I showed that the serum of vaccinated rabbits possesses a very marked protective power against infection by the coccobacillus of pneumo-enteritis. It was for the first time proved that independently of the antitoxic and bactericidal properties of serums, there exists another special property, the anti-infective property. This I conceived to be of the nature of a stimulant action on the part of the phagocytes.

It has already been stated in an earlier chapter that before the discovery of antitoxins Richet and Héricourt[2] had observed an immunising action of the serum of animals refractory to staphylococci. These observers were content with this demonstration and did not seek to penetrate more deeply into the mechanism of the action of their serum. For this reason when von Behring and Kitasato
[556] announced their discovery of antitoxic serums it was generally thought that the antistaphylococci serums were also antitoxic serums. The immunity against the micro-organism of the pneumo-enteritis of pigs taught us that here we might have to deal with quite a different matter. It was soon demonstrated that the serum from the immunised animal might in fact, without being antitoxic, present the same anti-infective property as in the case of pneumo-enteritis. That was first proved in the case of the experimental disease set up by Koch's cholera vibrio.

The reappearance of cholera in Europe in 1892 drew the attention of bacteriologists to this disease, and was the occasion of many new researches on immunity against the cholera vibrio. Several important works on this question were published by Pfeiffer[3], at this period director of the scientific staff of the Koch Institute at Berlin. He

[1] *Ann. de l'Inst. Pasteur*, Paris, 1892, t. VI, p. 289.
[2] *Compt. rend. Acad. d. sc.*, Paris, 1888, t. CVII, pp. 690, 748.
[3] *Ztschr. f. Hyg.*, Leipzig, 1894, Bd. XVI, S. 268.

obtained, in animals well immunised against the cholera vibrio, a serum endowed with a high anti-infective power but entirely without any antitoxic property. The guinea-pigs themselves, very resistant to the cholera peritonitis, were found, on the other hand, to be very susceptible to the minimum lethal dose of the cholera poison. The absence of antitoxic power in the fluids of the body taken in connection with a well-marked phagocytic reaction in a large number of cases of immunity, natural and acquired, has turned the scale in favour of the cellular theory. The impossibility on the part of those who maintain the purely bactericidal theory of the body fluids, to reply to the objections above mentioned has accentuated this favourable movement. Just at this moment, when the theory of phagocytes might be regarded to have obtained the rights of citizenship, a discovery was made which appeared to overturn it completely. I have mentioned more than once that the attempts of the partisans of the bactericidal theory of the body fluids have failed whenever it was necessary to give evidence of their action in the refractory animal. Instead of a destruction of the micro-organisms in these fluids, it was always found that they perished inside the phagocytes. These facts have even led to the manifestation of a desire to harmonise the humoral theory with the theory of phagocytosis. Denys, with certain of his collaborators, and Buchner and his pupils came to the conclusion that [557] the alexins are merely leucocytic products. As regards the theory of phagocytosis we have this section, who attribute an important function in healing and immunity to the emigration of the leucocytes towards, and their accumulation at the menaced spot. They admit that the leucocytes really represent the healing elements of the animal body; it is not, however, they say, their phagocytic functions which confer upon them this rôle but their power of secreting alexin. These bactericidal substances act outside the phagocytes—in the plasmas of the blood and of the exudations—and phagocytosis only intervenes at a later period and secondarily.

This new modification of the bactericidal theory of the body fluids has often been termed by Buchner a connecting bridge between the humoral theory and the cellular theory of immunity.

In the midst of this movement of conciliation, Pfeiffer[1] in 1894 published a work on the immunity of the guinea-pig against experimental cholera peritonitis. He maintains that here the

[1] *Ztschr. f. Hyg.*, Leipzig, 1894, Bd. XVIII, S. 1; cf. also Pfeiffer u. Issaeff, *ibid.*, 1894, Bd. XVII, S. 355.

destruction of the vibrios takes place without any co-operation on the part of the phagocytes and exclusively by means of the body fluids. The vibrios, before their complete destruction and solution in the fluids of the body, are transformed into granules, presenting the transformation to which we have given the name of Pfeiffer's phenomenon.

Several of Pfeiffer's pupils have confirmed his view in connection with the cholera vibrio, and have extended it to several other micro-organisms such as the typhoid coccobacillus. The destruction of the micro-organisms in these cases is brought about, according to Pfeiffer and his collaborators, not by the alexins of Buchner, but by a separate substance. The protective anti-infective serum contains it in an inactive state only; but immediately this serum is introduced into the body of a normal animal, the bactericidal substance is acted upon by the endothelial cells and becomes "active," capable of destroying a large number of vibrios. Pfeiffer has developed this theory more especially in an article published in 1896, entitled "Ein neues Grundgesetz der Immunität[1]." Pfeiffer's observation and his theory built upon it gave a new lease of life to the humoral theory and for some time many observers believed that the theory of phagocytosis
[558] was now finally overturned. Fränkel[2] announced, in a public address, that science in its progressive march has "discovered the methods of defence employed by the animal organism against its most dreaded enemies, methods which have nothing in common with phagocytosis, which act quite independently of the phagocytes and manifest an action so energetic that we may calmly eliminate all other factors." This view is based on the discovery of antitoxins and the bactericidal substance studied by Pfeiffer.

It will be readily understood that as soon as I learnt of the existence of a real extracellular destruction of micro-organisms I at once began to study it in order to find out its real importance amongst the phenomena of immunity. First of all, I examined Pfeiffer's phenomenon in connection with the cholera vibrio[3], and I was able to show that it was produced only under special conditions. The pre-existent phagocytes must be greatly injured before the cholera vibrios can be transformed into granules. Phagolysis (so I termed this transitory damage to the phagocytes) is indispensable for the

[1] *Deutsche med. Wchnschr.*, Berlin, 1896, SS. 97, 119.
[2] "Schutzimpfung und Impfschutz," Marburg, 1895.
[3] *Ann. de l'Inst. Pasteur*, Paris, 1895, t. IX, p. 433.

manifestation of Pfeiffer's phenomenon in the peritoneal fluid. When it is suppressed, by preparing the phagocytes by means of injections of various fluids, we find that, instead of Pfeiffer's phenomenon, phagocytosis is almost instantaneously produced. In positions where very few or no leucocytes are pre-existent, as in the subcutaneous tissue, Pfeiffer's phenomenon is never observed.

Even in the case of the cholera vibrio the extracellular destruction is observed, therefore, only in special cases. Most of the other pathogenic micro-organisms do not undergo this destructive process at all under conditions in which the cholera vibrio exhibits Pfeiffer's phenomenon in a marked degree. These facts appeared to justify me in the conclusion that the destruction of micro-organisms takes place in the animal body by means of soluble ferments, the result of phagocytic digestion. These ferments are found under the normal condition within these phagocytes and escape from them when they are destroyed or receive some transient injury. This conclusion was in flat contradiction to the theory and statements of Pfeiffer, who attributed an important function to the endothelial secretions. To settle this controversy I tried to obtain Pfeiffer's phenomenon outside the body, that is to say independently of any co-operation from the [559]
peritoneal endothelium. It is sufficient to add a little peritoneal lymph, rich in leucocytes, to the inactive anti-infective serum, to obtain in hanging drops the transformation of the cholera vibrios into granules.

Bordet[1], in my laboratory, repeated this experiment with the object of determining its essential mechanism. He succeeded in obtaining Pfeiffer's phenomenon *in vitro*, not only by adding peritoneal lymph from a normal guinea-pig to the specific serum, but also by adding to it a drop of fresh blood serum from the same animal. The analysis of the phenomena which take place under these conditions led Bordet to the following hypothesis. The destruction of micro-organisms in vaccinated animals takes place by the co-operation of two substances. One of these is Buchner's alexin which is found normally in the phagocytes; it sets up bacteriolysis properly so-called when it is enclosed within the leucocytes or after it has escaped from them at the time of phagolysis. To attain this end, however, the alexin needs the co-operation of another substance. This is the protective or sensibilising substance of Bordet. It circulates in the plasmas and carries a specific character which is absent

[1] *Ann. de l'Inst. Pasteur*, Paris, 1895, t. IX, p. 462; 1896, t. X, pp. 104, 193.

from the alexin. I need not here insist at any length on this theory, because it has already been sufficiently explained during the course of this work.

The data on the restricted part played by Pfeiffer's phenomenon and on its mechanism, above summarised, have been attacked by Pfeiffer and by several other observers, but they have received general confirmation, so that their accuracy can no longer be in doubt. Objections were also raised to Bordet's view of the mechanism of bacteriolysis. Thus, Abel has criticised it in the following argument[1]: "In spite of the soundness and the boldness of the majority of Bordet's statements on the importance of the various factors, and especially of the leucocytes in immunity, it cannot be doubted that later researches will modify and correct his interpretations which we, in Germany, do not accept in their full extension. Up to the present, the victory in the various rounds has always been with Pfeiffer, whose researches, solid and exempt from bias, have made him, to use a sporting expression, the 'favourite' with all those who follow atten-
[560] tively the international contest in the arena of the problem of immunity." Abel is certainly a highly esteemed bacteriologist, but he is not a good prophet, and he assumes a mistaken attitude in looking at the subject from a "national" point of view[2]. In Germany much interest is taken in scientific movements and, very naturally, original and new theories are there criticised and discussed. But that does not justify one in putting forward against an opinion the statement that it is not accepted in Germany. In this country, so rich in scientific work, we find partisans of the most opposite views. In any case, in the conflict between Pfeiffer on the one hand, and Bordet and myself on the other, things have not turned out as Abel predicted. The two substances which act in the destruction of the micro-organisms are now accepted by the whole world. The intimate relations between the alexins and the leucocytes are equally recognised

[1] *Centralbl. f. Bakteriol. u. Parasitenk.*, Jena, 1896, I^te^ Abt., Bd. xx, S. 766.

[2] It would clearly be wrong to take one's stand, in a purely scientific question, on a national point of view. But it is a still greater mistake to look at matters, in the investigation of problems which concern science only, from a personal point of view. This, however, is what has happened several times in the discussion of phagocytosis. Certain discontented students have attempted to avenge themselves by publishing works and criticisms directed against the theory of phagocytosis. Having no doubt as to the motive for these publications I consider myself fully justified in not referring to them in this book, in which I have taken an exclusively scientific point of view, and in which I have endeavoured to weigh as carefully as possible all criticisms and objections that have been directed against me.

by very many observers. The fact that the alexins are confined within phagocytes has been confirmed by several observers, and has received a very convincing proof from Gengou's experiments on the comparative action of the serum and blood plasma against micro-organisms. The existence of phagolysis, denied at first by some observers, has been verified by others and can now no longer be doubted.

The relations between the sensibilising substance and the phagocytes are less easily grasped than are those between the alexins and the leucocytes. Nevertheless, the experiments made by Pfeiffer and Marx[1], have led these observers to recognise that the former arise from the spleen, the lymphatic glands, and the bone marrow, that is to say, organs which are pre-eminently phagocytic. This result has been confirmed by Deutsch and must be regarded as definitely settled. All the data collected in recent years have, therefore, confirmed the view that the destruction of micro-organisms in the refractory animal presents itself as a special example of their absorption by formed elements. This truth was so fully recognised [561] in our laboratory that the analogy between bacteriolysis and the destruction of animal cells was looked upon as quite natural and evident. Bordet had for some years past observed that the blood serum of certain animals presented a marked analogy in its agglutinative property in regard to micro-organisms and in that against red blood corpuscles. In 1898, studying the fate of the spirilla of the goose in the peritoneal cavity of guinea-pigs (see Chapter VI), I observed that these micro-organisms underwent the same changes both within and outside the phagocyte; this fact appeared to me to be in perfect harmony with the whole of our knowledge concerning the absorption of formed elements and on intracellular digestion.

Bordet[2], prepared by his preceding researches on the agglutination of the red blood corpuscles, set himself to study the fate of the red corpuscles in the animal body. He easily established a close relationship between the development of the bacteriolytic property and the haemolytic power of the serum of animals prepared by repeated injections of bacteria and of blood. His results were soon (January, 1899) confirmed by Ehrlich and Morgenroth[3], who supplemented them with the important statement that Bordet's sensibilising substance, or

[1] *Ztschr. f. Hyg.*, Leipzig, 1898, Bd. XXVII, S. 272.
[2] *Ann. de l'Inst. Pasteur*, Paris, 1898, t. XII, p. 688; 1899, t. XIII, p. 273.
[3] *Berl. klin. Wchnschr.*, 1899, S. 6.

intermediary substance (E. and M.), has the property of attaching or fixing itself to the red blood corpuscles.

The works on haemolysis, carried out during the last three years by Ehrlich and Morgenroth on the one hand, and by Bordet on the other, have allowed us to extend our study of the mechanism of the action of the two substances on micro-organisms and on animal cells. Ehrlich has extended his ingenious theory of antitoxins to the bacteriolytic substances, which he regards as side-chains detached from the cells and capable of absorbing the toxins. In a series of remarkable investigations, most of them carried out in collaboration with Morgenroth, Ehrlich has developed his theory which attempts to offer an account of the essential mechanism which presides over the destruction of micro-organisms and over the neutralisation of their poisons. This theory is at present in full swing of development. Some of his points contradict several of the conclusions in Bordet's works. Whilst the latter maintains that the sensibilising substance becomes fixed as
[562] a mordant, Ehrlich regards it as entering into chemical combination with the molecular group of the micro-organisms and of the animal cells. According to Bordet, the alexin of the same species of animal is always the same substance. Ehrlich energetically maintains the plurality of the alexins, to which he gives the name of complements.

This controversy has caused a most interesting exchange of views and has led to experiments which are remarkably ingenious; but it must be admitted that as yet all the points in dispute are not definitely settled. It is evident that we have here a new line of research which promises most fruitful results for science.

We have described in various chapters of this work the fundamental elements of Ehrlich's theory. Many think that this theory is, in principle, antagonistic to the theory of phagocytosis, but we have already observed that this view cannot be accepted. It is true that Ehrlich maintains that the bacteriolytic and cytotoxic ferments which we have called *cytases* (alexins or complements) circulate in a state of solution in the blood plasma, whilst, according to the theory of phagocytosis, they are found under normal conditions inside phagocytes. But this view has nothing to do with the basis of the theory of receptors, or of Ehrlich's side-chain theory, according to which the antitoxin and certain other antibodies (intermediary substance) are regarded as products detached from cells having an affinity for the toxins and the microbial products.

The theory of phagocytosis seeks to establish the part played by these cells in the destruction of micro-organisms. It maintains that the vital manifestation of the phagocytes, irritability, mobility, and voracity, constitutes an essential factor in ridding the animal of micro-organisms, because the true bactericidal ferment is contained within the phagocytes, except in cases of phagolysis. The destruction of the micro-organisms follows the laws which govern the absorption of formed elements in general. This absorption, finally, is the work of two soluble digestive ferments, one of which (fixative) is readily excreted by the phagocyte into the plasmas of the blood and exudations. The theory of phagocytosis seeks to establish these principles with the greatest possible exactness, but it has not yet ventured to penetrate more deeply into the phenomena of intracellular digestion which are confounded with the action of soluble ferments in general. This problem is still far from being satisfactorily solved.

In spite of very numerous objections, of which the principal ones [563] have already been mentioned, the theory of phagocytosis, within the limits indicated, so far from being overturned, has become more and more consolidated, thanks to the numerous observations made since its foundation. It is for this reason that the opposition has calmed down of late years and that in many works the opinions expressed have become more favourable to the rôle of phagocytosis in immunity.

Soon after the Congress of Hygiene in 1891, the Pathological Society of London devoted several meetings to a discussion of the question of immunity. Many eminent observers took part in these debates, which were, in general, favourable to this theory of phagocytosis[1].

At the International Congress of Hygiene, held at Budapest in 1894, the question of immunity was again discussed. Buchner[2] made a report in which he specially insisted on the leucocytic origin of the alexins, regarding this fact as particularly capable of reconciling the bactericidal property of the body fluids with the theory of phagocytosis. The alexins, however, secreted by the leucocytes, must, it was assumed, carry out their principal function in the plasmas of the blood and exudations. Phagocytosis would only intervene secondarily for the purpose of ingesting the micro-organisms which had been already killed or seriously injured by the alexins of the body fluids.

[1] *Brit. Med. Journ.*, London, 1892, Vol. I, pp. 373, 492, 591, 604. A very short summary of this discussion was given in the *Deutsche med. Wchnschr.*, Leipzig, 1892, S. 296.

[2] *München. med. Wchnschr.*, 1894, S. 717.

In his last summary of the question, presented to the International Congress of Medicine at Paris in 1900, Buchner[1] maintains his theory of leucocytic secretions. But he already takes one step more towards the theory of phagocytosis, at least as regards natural immunity. He consents to accept the fact "that phagocytic activity is in many cases of decisive importance in overcoming the infective processes, especially in those cases in which the secreted alexins were unable to bring about more than a temporary attenuation of the vital functions of the bacteria. Under these conditions the bacteria could only be modified in so far as their chemical functions were transformed into a latent state, from which they would be ready to regain their full vital activity should it happen that the phagocytes were not there to prevent them from doing so." In any case this view is
[364] widely removed from the old theory, according to which phagocytes were regarded as capable of ingesting dead and inoffensive bacteria only.

A second adversary of the theory of phagocytosis, von Behring[2], gives a place to this theory not only in certain examples of natural immunity but even in some cases of acquired immunity, e.g. in the immunity of sheep vaccinated against anthrax, an example I have already cited in Chapter VIII (cf. *supra*, p. 242).

It would take too long to describe the change of opinion on the theories of immunity that has taken place during recent years. I will content myself with citing certain examples which shall be taken from the works of declared adversaries of the theory of phagocytosis. Thus, Flügge, who early declared against the cellular theory completely and categorically and at the same time argued strongly in favour of the humoral theory, has been gradually led to depart from his first position. We may follow the steps of his conversion in the different editions of his *Outlines of Hygiene*. In the first edition published in 1889 he expresses himself in the following manner[3]: "Recent researches indicate the probability, however, that the phagocytes in by far the greater majority of cases seize the infective agents which, already dead, are not in a condition suitable for the performance of a defensive function. On the other hand, it is proved that the blood and blood plasma of warm-blooded animals possess the property of destroying, very quickly, enormous numbers of pathogenic bacteria,"...etc. In

[1] *München. med. Wchnschr.*, 1900, S. 1193.
[2] *Encyclop. Jahrbücher*, Wien, 1900, Bd. IX, S. 203.
[3] "Grundriss der Hygiene," Leipzig, 1889, S. 487.

the fourth edition of the same work, published in 1897, we find at the corresponding place the following passage[1]: "Recent researches indicate the probability, however, that the theory of Metschnikoff... is not in a position to offer a complete explanation of the process of immunity." This passage is followed by a somewhat conciliatory and eclectic development of the theory.

Let us take as a second example Günther's *Introduction to the Study of Bacteriology*, widely read both in the original and in translations. In the first edition published in 1890[2] the theory of phagocytosis is curtly dismissed as "being incapable of withstanding criticism." In the fifth edition of the same work, however[3], published in 1898, this theory is no longer treated thus summarily. It is given [565] a place amongst the theories of immunity and an attempt, similar to that made by Buchner, is made to reconcile it with the humoral theory.

A change in the same direction may also be observed in Charrin's view. In the first edition of his *Pathologie générale infectieuse*, this observer[4] had already taken an eclectic view on this question of the theories of immunity. But the function which he assigns to the phagocytes is subsidiary and secondary, whilst to that of the humoral properties is assigned a position of primary importance. In the second edition of the same work, which appeared seven years later[5], the importance of phagocytosis is recognised in a much larger measure, as may be gathered from the following passages: "For my part, I have always accepted phagocytosis: at the same time I have always accepted the existence of special humoral properties. As early as 1888 I showed, *in vivo*, that the germs are modified outside the cells; but I did not know from what groups of anatomical elements these properties were derived, I exaggerated their importance and it is the decision of this origin and this importance that renders it possible to reconcile the two theories" (p. 250). "After all, the defence rests upon these two great processes or cellular activities, phagocytosis in the first line, and then humoral influences, some of them bactericidal and injurious to the living germ, others antitoxic and injurious to their secretions" (p. 253).

Whilst the theory of phagocytosis has been consolidated by the

[1] "Grundriss der Hygiene," Leipzig, 4te Aufl., 1897, S. 507.
[2] "Einführung in das Studium der Bakteriologie," Leipzig, 1890, S. 146.
[3] "Einführung in das Studium der Bakteriologie," 5te Aufl., 1898, S. 275.
[4] "Traité de médecine" de Charcot, Bouchard, et Brissaud, 1891, t. I, pp. 219—230.
[5] "Traité de médecine...," 2e éd., 1898, t. I, pp. 250—254.

demonstration: (1) that the phagocytes, in cases of immunity, ingest and destroy the living and virulent micro-organisms without the latter needing to be previously deprived of their toxins; (2) that the phagocytes absorb toxic substances; (3) that the phagocytes contain bactericidal cytases and produce fixatives; the humoral theories, in spite of all the efforts made to defend them, could never be developed as theories that were in the slightest degree of general application. Certain observers who from the first were very sympathetic to the humoral theories have attempted to give a complete summary of these properties. Thus, Stern[1] and later Frank[2] have published reports drawn up with great care and in a very impartial spirit on the works treating of the properties of the body fluids
[566] and the part they play in immunity. This is how they sum up the question. Stern came to the conclusion that it is impossible "to demonstrate at all regularly the existence of relations between the bactericidal action of the blood and immunity in all the infective diseases. In some cases, however, these relations are so marked that, for these examples, a causal bond between the two factors is extremely probable." Frank expresses himself in the following manner: "It follows most clearly that the immunity of an animal—immunity innate or acquired—corresponds with the bactericidal property of the blood in certain exceptional cases only. The only animal, absolutely susceptible to anthrax and whose blood is entirely without any bactericidal power, that it is at present possible to cite, is the mouse." "The bactericidal action of the blood serum is undoubtedly a fact of great biological importance; but equally certainly it cannot be the general cause of immunity, whether innate or acquired."

An attempt was made to give fresh life to the humoral theory, either by assuming that the bactericidal substance is nothing but the eosinophile or pseudo-eosinophile secretion of the leucocytes (Kanthack), or by supposing that for the destruction of micro-organisms in the animal body the intervention of the agglutinative substance dissolved and distributed in the body fluids is essential (Max Gruber). These two views were put forward in a tentative form and as preliminary communications only; there is no possibility

[1] *Centralbl. f. allg. Path. u. path. Anat.*, Jena, 1894, Bd. v, S. 212.

[2] Lubarsch u. Ostertag's "Ergebnisse d. allg. Path. u. path. Anat.," Wiesbaden, 1895, I. Abt., S. 384.

of raising them to the dignity of theories, and of late years they have not been upheld.

It cannot be denied that not one of the humoral theories has been able to retain its position or to stand against the numerous facts that have been accumulated during recent years.

This extraordinary discrepancy between the bactericidal power of the body fluids and immunity is explained by the circumstance that the microbicidal substances exist in the living animal within phagocytes and only escape from them when these cells have been injured. The fact, so well demonstrated by Gengou, that the blood plasma is without any bactericidal power has given the final blow to the microbicidal theory of the body fluids and it can no longer be maintained.

The humoral theories, based on the antitoxic and protective power of the body fluids, can claim only a very restricted application. These properties are met with in acquired immunity only, and even there are not constant. Many cases of acquired immunity against micro- [567]
organisms are unaccompanied by any antitoxic power, and in several examples of this immunity the body fluids do not exhibit any protective power.

There is only one constant element in immunity, whether innate or acquired, and that is phagocytosis. The extension and importance of this factor can no longer be denied.

It is clearly proved that phagocytes are susceptible cells which react against morbific agents, whether organised or not. These cells ingest micro-organisms and absorb soluble substances. They seize microbes whilst these are still living and capable of exercising their noxious effect and bring them under the action of their cellular contents, which are capable of killing and digesting the micro-organisms or of inhibiting their pathogenic action. Phagocytes act because they possess vital properties and a faculty of exerting a fermentative action on morbific agents. The mechanism of this action is not yet definitely settled, and we can foresee that for future researches there will be a vast and fertile field to be reached by pursuing this path.

The present phase of the question of immunity constitutes one stage only in the development of biological science and one which is capable of many improvements.

[568]

CHAPTER XVII

SUMMARY

Means of defence of the animal against infective agents.—Absorption of micro-organisms.—Phagocytes, and their function in inflammation.—The action of phagocytes in the absorption of micro-organisms.—The cytases, phagocytic ferments.—The cytases are closely bound up with the phagocytes.—The fixatives and their function in acquired immunity.—The fixatives are excreted by the phagocytes and pass readily into the fluids of the body.—Essential mechanism of the action of the fixatives.—Adaptation of phagocytes to destroy micro-organisms in acquired immunity.—Difference between the fixatives and the agglutinins.—Antitoxins and their analogy with the fixatives.—Hypotheses as to the origin of antitoxins.—Cellular immunity is a fact of general import.—Susceptibility and its rôle in immunity.—Applications of the theory of immunity to medical practice.

When an animal remains unharmed in spite of the penetration of infective agents it is said to be immune to the diseases usually set up by these agents. This idea embraces a very great number of phenomena which cannot always be sharply separated from allied phenomena. On the one hand, immunity is closely connected with the process of cure, on the other, it is related to the disease. An animal may be regarded as unharmed if the penetration of a very dangerous virus sets up merely an insignificant discomfort. Nevertheless, this discomfort is accompanied by morbid symptoms, though they may be very slight. It is useless and impossible to set up any precise limits between immunity and allied states.

Immunity presents great variability. Sometimes it is very stable and durable; in other cases it is very feeble and transient. Immunity may be individual or it may be generic. It may be the privilege of a race, of a species.

Immunity is often innate, as is the case of the immunity which is called natural. But it may also be acquired. This last category of immunity may be developed either by natural means, after an attack of an infective disease, or as a result of human intervention. The

principal means of obtaining artificial acquired immunity consists in the inoculation of viruses and of vaccines.

Immunity is a phenomenon which has existed on this globe from [569] time immemorial. Immunity must be of as ancient date as is disease. The most simple and the most primitive organisms have constantly to struggle for their existence ; they give chase to living organisms in order to obtain food, and they defend themselves against other organisms in order that they may not become their prey. When the aggressor in this struggle is much smaller than its adversary the result is that the former introduces itself into the body of the latter and destroys it by means of infection. In this case it takes up its abode in its adversary in order to absorb the contents of its host and to produce within it one or more generations. The natural history of unicellular organisms, both vegetable and animal, often presents to us these examples of primitive infection.

But infection also has its counter. The attacked organism defends itself against the little aggressor. It protects itself by interposing a resistant membrane, or it uses all the means at its disposal to destroy the invader. As a very large number of organisms, in order to obtain nourishment, are obliged to submit their food to digestion by various chemical substances, they utilise these substances in the struggle against the infective agents. They digest them whenever they are able to do so.

One of the most primitive of organisms, the plasmodium of the Myxomycetes, which is composed of formless protoplasmic masses intermediate between lower animals and plants, ingests foreign bodies of various kinds. It often happens that it incorporates numerous bacteria which are growing alongside it on rotten wood or elsewhere. The plasmodium allows them to live for some time within its digestive vacuoles. But in the end it digests them by means of its soluble ferments, substances intermediate between pepsin and trypsin. Owing to this digestive power the plasmodia are not attacked by bacterial infections.

This example, taken from amongst the most simple organisms, may serve as a prototype for the phenomena of immunity in general. At the commencement of the study of this remarkable property of so many living organisms it was thought that the pathogenic micro-organisms encountered, within the refractory organism, a medium which did not allow them to live, either because of the absence of certain nutritive substances indispensable for their existence or

because it contained some substance injurious to micro-organisms.
[570] Very numerous and detailed researches have demonstrated the incorrectness of these hypotheses. There are, of course, certain pathogenic micro-organisms which are very exacting as regards the medium in which they will grow. Some will develop only in the presence of particular substances, whilst others are extremely sensitive to the slightest traces of poisons. These, however, are quite the exception. The great majority of pathogenic micro-organisms belonging to the group of bacteria readily adapt themselves to all kinds of culture media, and most of them live and develop freely in the blood or other fluids of refractory organisms. This, therefore, is not the cause of the immunity in such organisms. The cause must be sought for amongst factors more closely connected with life.

Wishing to penetrate more deeply into these phenomena the hypothesis was put forward that the unharmed organism got rid of the infective micro-organisms by expelling them to the outside along with the excreta. It was maintained for a considerable time that the animal organism possessed the means of causing pathogenic bacteria to pass into the kidneys, whence they were eliminated by the urine. It had to be acknowledged, however, that this elimination never takes place in cases of immunity, and only comes into operation when the animal is ill and the integrity of the renal filter is impaired.

The infective micro-organisms, after they have entered into the unharmed organism, remain there for a longer or shorter period, and perish without being expelled. This disappearance of the micro-organisms takes place by the same mechanism that rids the plasmodium of those bacteria which it has managed to ingest during its slow peregrinations over dead leaves or rotten wood. The micro-organisms are absorbed into the refractory organisms as the result of a true act of digestion. It is very remarkable that the gastro-intestinal ingestion, so well provided with means of rendering the most varied aliments soluble, is generally incapable of digesting pathogenic or other micro-organisms. It is very rare to meet with soluble ferments of the intestinal canal which are capable of digesting microscopic organisms, especially bacteria. Consequently this organ, so rich in digestive diastases, is generally inhabited by a large number of bacteria and other micro-organisms.

Even in animals whose food contains large numbers of micro-

organisms, *e.g.* the larvae of flies, the digestive juices are powerless to destroy them. Nevertheless, there are organisms which feed exclusively, or almost exclusively, on bacteria and which are quite capable of digesting them. These are the Protozoa, such as the *Amoebae* and certain Infusoria, which, without any trace of a [571] digestive tube, easily bring about this result. *Amoebae* can be grown on the surface of agar by taking care to sow along with them bacteria for their nourishment. It is only necessary to give them a single species of micro-organism, and this may be selected from the pathogenic forms, such as the cholera vibrio or the *Bacillus coli*. The *Amoebae* ingest a number of these bacteria in the living state. They then kill them and digest them in their digestive vacuoles which contain, along with a little acid, a ferment belonging to the trypsin group, the amoebodiastase.

The bodies of lower and higher animals, alike, are very rich in elements which closely resemble the *Amoebae*. Sometimes these are to be found in the epithelial cells of the digestive canal which put out protoplasmic processes for the purpose of seizing food and transferring it to their interior, where it is submitted to the action of digestive ferments. Sometimes they are the cells disposed between the body wall and that of the intestinal canal, which float freely in the fluids of the body or are more or less fixed in the interstitial tissue. The animal kingdom presents a great variety of these amoeboid elements, known under the general name of phagocytes (cells capable of devouring solid bodies). One of the most primitive arrangements of phagocytes is met with in *Ascaris* and its allies belonging to the group of the Nematoda. All the organisation that these round worms possess consists merely of four, or a few more, enormous cells attached to the body wall. These are phagocytes which push out processes of enormous length, capable of exploring the whole of the internal cavity of the body.

The majority of phagocytes circulate in the lymph and blood and pass into the exudations. These white corpuscles have a comparatively uniform structure in the Invertebrata and present themselves as small cells with a nucleus and a protoplasm capable of amoeboid movements. In the Vertebrata we meet with two great categories of white corpuscles, of which one group resembles those of the Invertebrata in that they also possess a single large nucleus and an amoeboid protoplasm. These are the macrophages of the blood and of the lymph, and are intimately connected with the macrophages of such organs as

the spleen, lymphatic glands, and bone marrow. Another group of white corpuscles in the Vertebrata is made up of small amoeboid cells which are distinguished by having a nucleus which, although single, is divided into several lobes. These are the microphages
[572] whose chief peculiarity, the multi-lobed form of the nucleus, must be regarded as an adaptation for the purpose of passing as rapidly as possible through the walls of capillaries and small veins.

The diapedesis of the white corpuscles, their migration through the vessel wall into the cavities and tissues, is one of the principal means of defence possessed by an animal. As soon as the infective agents have penetrated into the body, a whole army of white corpuscles proceed towards the menaced spot, there entering into a struggle with the micro-organisms. Aided by the special form of their nucleus the microphages are the first to pass through the walls of the vessels. Each of the several small lobes, into which the nucleus and its protoplasm is divided, passes readily through the minute orifices between the endothelial cells of the vessels. The macrophages follow the microphages and become mixed in greater or less numbers with the exudations. But it is not micro-organisms only which set up this inflammatory reaction accompanied by the emigration and the accumulation of leucocytes. The introduction of inert bodies and of aseptic fluids brings about the same result. The phagocytes are, as a matter of fact, endowed with a special susceptibility, which enables them to perceive exceedingly small changes in the chemical or physical composition of the medium that surrounds them.

The leucocytes, having arrived at the spot where the intruders are found, seize them after the manner of the *Amoebae* and within their bodies subject them to intracellular digestion. This digestion takes place in the vacuoles in which usually is a weakly acid fluid which contains digestive ferments; of these a very considerable number are now recognised.

Just as the *Amoebae* and the Infusoria make a choice from amongst the small organisms that surround them, so the leucocytes choose bodies which are best suited to their use. The macrophages seize by preference animal cells such as the blood corpuscles, the spermatozoa, and other elements which are derived from animals. Among the infective micro-organisms the macrophages have a predilection for those that set up chronic diseases such as leprosy, tuberculosis, and actinomycosis and also for those which are of animal nature. Into this last category come the amoeboid parasities of malaria, Texas

fever and the *Trypanosomata*. The macrophages can also ingest the bacteria of acute diseases, but, save in exceptional cases, their intervention is of little moment.

The microphages, on the other hand, appear to play their part [573] specially in acute infections. Their intervention against animal cells is *nil*, or almost so. Thus they rarely seize the red corpuscles of the same or of a foreign species of animal. They also appear to be repelled by parasites of animal origin and by certain bacteria which set up chronic diseases. Whilst the macrophages seize the bacilli of leprosy with great avidity, the microphages ingest them only exceptionally.

The morphological and physiological differences between the two great categories of mobile phagocytes (leucocytes), correspond to differences in the composition of their soluble ferments. Just as the *Amoebae* digest their prey by means of their amoebodiastase, a soluble ferment of the group of trypsins, so the white corpuscles submit the foreign bodies ingested by them to the action of what are now known as cytases. These cytases (alexins or complements of other writers) are soluble ferments which also belong to the trypsin group. They act in a medium which is feebly acid, neutral, or feebly alkaline, and, like the amoebodiastase, they are distinguished by a great sensitiveness to heat. When the cytases are contained in fluids, a temperature of 55°—56° C. destroys them rapidly and completely. When they are found in organs reduced to the state of an emulsion, their sensitiveness diminishes and it is necessary to raise the temperature to 58°—62° C. in order to destroy their activity.

Bordet maintains that the cytases are very different in the various species of animals, but that in the same species only one cytase exists. Ehrlich and Morgenroth, on the other hand, hold that the same serum contains several, sometimes many, different cytases. This question is too difficult to be definitely solved at present. It appears to me very probable that there exist, in the same species of animal, two different cytases. One of these, the macrocytase which is found in the lymphoid organs and in the serum of the blood, acts more particularly on animal cells. Thanks to this substance an extract or maceration of the spleen, omentum or lymphatic glands dissolves the red blood corpuscles more or less readily; these extracts and macerations, however, are incapable of destroying bacteria. When the macrophages seize the nucleated blood corpuscles they digest them completely, not sparing even the nucleus, so resistant

[574] to attack, but when the same phagocytes ingest such micro-organisms as are most easily digested, such as the cholera vibrio, their action is feeble. The vibrios, without any transformation into granules, remain alive for some time and are destroyed and digested with very great difficulty. The cytase of the microphages, or microcytase, is distinguished by other properties. It destroys and digests easily many micro-organisms, but has little or no action upon the red blood corpuscles and other animal cells. The exudations which are rich in macrophages, such as those of the lymphoid organs, are not at all or only slightly bactericidal, but exhibit a solvent action on red blood corpuscles. On the other hand, the exudations, which are composed in great part of microphages, leave red blood corpuscles intact, but readily destroy micro-organisms. Similar properties distinguish the bone marrow, extracts and suspensions of which do not dissolve red corpuscles, but attack micro-organisms. Now, we know that the bone marrow is the principal seat of origin of the microphages.

Even after the addition of some of the specific fixative to the microphagic exudations no solution of the red corpuscles is produced, which demonstrates most clearly that the microcytase is really incapable of attacking these animal cells.

We are, therefore, compelled to accept the existence of two different cytases, of which one (the macrocytase) acts specially upon elements of animal origin, and the other (the microcytase) acts principally on micro-organisms. The indication of any more detailed differentiations is impossible in the present state of our knowledge.

There are certain ferments which, during the life of the cells which produce them, pass readily into the surrounding fluids. For instance, sucrase can be recovered without difficulty from the culture fluid of moulds and yeasts. The ferments of the intestinal digestion also pass with great facility into the secreted fluids. Other soluble ferments, on the other hand, remain very closely bound up with the cells which manufacture them. Thus the zymase of the yeasts can only be freed from the cells of these fungi with great difficulty, under the influence of great pressure and under conditions which profoundly alter the cell. The proteolytic ferment of the yeast is also very adherent to the cells of these organisms. The fibrin-ferment, or plasmase of the white corpuscles, is not secreted by these cells so long as they are quite intact. But it is sufficient to subject them to

unfavourable conditions of existence to cause them to throw it out from their bodies. The leucocytes, when removed from the animal, [575] undergo a deterioration which soon leads to the deposition around them of filaments of fibrin.

The cytases must also be grouped with the soluble ferments which are not thrown off by the phagocytes so long as these remain intact. Immediately these cells are injured, however, they allow a part of their cytases to escape. In the blood, withdrawn from the animal, the white corpuscles allow the plasmase to pass into the fluid, where it sets up the coagulation of the fibrin and the formation of a clot. At the same time these cells give up some of their cytases which communicate to the serum its haemolytic and bactericidal properties. This fact is of the highest importance in connection with the question of immunity. The best demonstration of this has been furnished by a comparison of the bactericidal power in the different parts of the body and in the body fluids extracted from the animal.

When micro-organisms are introduced into those situations in the refractory animal which contain pre-existent leucocytes, the leucocytes, under the influence of the shock, undergo serious lesions, accompanied by the throwing out of the cytases. Under these conditions the least resistant micro-organisms (such as the cholera vibrio) exhibit undeniable signs of deterioration: they become transformed into granules and may even die in greater or less numbers. When, however, the leucocytes are well protected and withstand the injection of the micro-organisms without being profoundly altered, the extracellular destruction of the micro-organisms does not take place. On the contrary, a very rapid phagocytosis is produced which brings about the death and intracellular digestion of these micro-organisms. Under these conditions vibrios are also transformed into granules and perish, but only within the leucocytes. The phenomena I have just mentioned are brought about in the peritoneal cavity and in the blood vessels of refractory animals, that is to say, in situations rich in leucocytes.

In the subcutaneous tissue, in the fluids of oedemas and in the anterior chamber of the eye of these same refractory animals, the phenomena are very different. As in these situations there are no pre-existing leucocytes or their number is insignificant, the micro-organisms introduced do not suffer serious injury; they continue to live up to the moment when the leucocytes, having come up as the result of the inflammatory reaction, seize them alive, kill them,

[576] and digest them within their substance. Just as it is easy, in situations populated by pre-existing leucocytes, to suppress the extracellular destruction of the micro-organisms by preserving the phagocytes against injury or phagolysis, so this same extracellular destruction is easily set up in situations where leucocytes are absent. When, after exudations rich in leucocytes have been injected into the subcutaneous tissue, we introduce micro-organisms which are not very resistant, such as the cholera vibrio, it is observed that these vibrios are destroyed outside the cells, having first been transformed into granules.

There can be no doubt as to the conclusion to be drawn from these various experiments. The microcytase is the substance which transforms the vibrios into granules. It is within the microphages, when they remain intact, that the vibrios undergo transformation. When, on the other hand, the microphages are injured and allow the microcytase to escape, the transformation of the vibrios into granules and their partial destruction take place in the plasmas outside the phagocytes.

This conclusion is supported by comparative researches on the bactericidal power of the serum and of the blood plasma outside the animal. It is true that it is impossible to prepare a fluid which shall in all respects be comparable to the plasma of the circulating blood. There is, however, always a means of obtaining outside the animal a fluid which approaches much more closely to blood plasma than does serum. Gengou succeeded in preparing in tubes coated internally with paraffin a fluid which coagulates very tardily, and which contains very little fibrin-ferment. This fluid is found to be much less bactericidal than is the blood serum of the same animal. It is, indeed, often found to be entirely without bactericidal power, whilst the corresponding serum is capable of destroying a large number of micro-organisms.

In the phenomena of the absorption of cells also a great number of facts are met with which demonstrate that the macrocytase escapes from the macrophages at the moment of their phagolysis only. For example, the extracellular solution of the red corpuscles takes place easily in the peritoneal fluid of animals prepared by a previous injection of the same corpuscles. When the leucocytes of the peritoneal cavity are abandoned to their fate, a marked phagolysis is produced and consequently a solution of the red corpuscles in the fluid itself. When, on the other hand, phagolysis

is prevented, the macrophages remaining intact do not allow their [577]
macrocytase to escape and the solution of the red corpuscles takes place almost exclusively inside the phagocytes.

In certain animals the blood serum arrests the movements of their own spermatozoa at once, whilst these remain quite motile in the animal itself. This is due to the fact that the immobilising macrocytase is contained within the macrophages and does not escape from them so long as these cells remain intact. When, in such animals, their own spermatozoa are introduced into the subcutaneous tissue, they remain motile for a long time; when, on the contrary, the spermatozoa are injected into the peritoneal cavity, where the leucocytes have not been prepared, phagolysis is produced at once and the spermatozoa become motionless immediately.

As all these data agree in demonstrating that the uninjured phagocytes retain the cytases—which remain within them, and are not found in the surrounding fluids,—we can readily understand the reason for the differences between the phenomena of immunity and the bactericidal power of the body fluids. The rat's serum is capable of destroying a large number of anthrax bacilli, although these rodents are certainly susceptible to anthrax. The reason for this is that in the serum of the rat the bacilli are destroyed by the microcytase which is set at liberty, whilst in the body of the animal it remains enclosed within the bodies of the living microphages. So long as these cells exhibit a negative chemiotaxis against the anthrax bacillus, the micro-organism remains in the plasma, where it is not interfered with. Thanks to this, multiplication of the bacilli goes on in the body of the animal, the micro-organism killing it after becoming generalised in the blood and in the organs. The susceptibility of the leucocytes is, then, the cause of the death of the rats from anthrax, the organism of these rodents being unable to take advantage of its richness in bactericidal microcytase.

Another paradoxical fact is met with in guinea-pigs immunised against Gamaleia's vibrio (*Vibrio metchnikovi*). As demonstrated by von Behring and Nissen, the blood serum of these guinea-pigs is very bactericidal for the vibrio in question. A contact of less than an hour is quite sufficient to destroy large numbers of the micro-organisms. Nevertheless, when a small dose of a culture is injected subcutaneously into these hypervaccinated guinea-pigs, the vibrios remain alive for several days, up—indeed, to the moment when they are ingested and destroyed by the leucocytes which come up in large

numbers to the menaced spot. This apparent contradiction is easily explained by the fact that it is in the serum only that the vibrios encounter the microcytase, which has escaped from the microphages at the time of the formation of the clot and the separation of the serum.

[578] Alongside those cases in which the serum of susceptible animals is found to be very bactericidal, examples are not wanting where the blood and the serum of refractory animals are entirely without this power. For instance, the pigeon is refractory to Pfeiffer's influenza bacillus, but the blood of the pigeon forms the best culture medium for this micro-organism. The dog is refractory to the anthrax bacillus, against which the blood serum of the same animal is not at all bactericidal. The cause of this absence of parallelism between immunity and the bactericidal power of the serums must be sought in the difficulty with which the cytases escape from the leucocytes, and also in the modifications which they may undergo, once they are distributed in the fluids.

In cases of natural immunity, the cytases rid the animal of the micro-organisms without the slightest observable co-operation on the part of other soluble ferments. It is impossible to settle definitely even the question whether, in animals which enjoy this innate immunity, there exists, alongside the microcytase, any ferments which come to its aid. The conditions are quite otherwise in a very large number of cases of acquired immunity. Here it is found, as a fairly general rule, that in addition to the microcytases there exist other substances whose rôle in the defensive action offered by the animal against micro-organisms is very important. These substances are fixatives which co-operate in a remarkable fashion with the bactericidal action of the cytases; but whilst these latter injure the bacterial cell directly, the fixatives do not interfere with its life. The bacteria, permeated by fixatives, may even continue to reproduce themselves and, under certain conditions, to invade the animal. The fixatives, then, are not bactericidal, but by fixing themselves upon the micro-organisms they render them much more susceptible to the bactericidal action of the microcytases. These latter are further distinguished, in several other respects, from the cytases. The fixatives must also be classed with the group of soluble ferments, but they resist much higher temperatures than those which destroy the cytases. Whilst the latter are quite destroyed at 55° C., the fixatives, to be completely altered, must be heated to beyond 60° C. and even 65° C. On the other hand, the fixatives are distinguished

by a high specificity which is never observed in the cytases. The majority of the fixatives are incapable of fixing themselves upon more than a single species of bacteria or upon a single class of animal cells, and only certain of them can fix themselves upon [579] allied species or cells, such as the red corpuscles of several species of animals. In these cases, too, there exists a sharp quantitative difference between the fixation on the different formed elements. The same microcytases are, on the other hand, able to attack all kinds of micro-organisms, and the same macrocytases attack all kinds of animal cells.

We have seen that the cytases correspond to the zymase and to the proteolytic diastase of the yeasts in the sense that all these soluble ferments adhere with tenacity to the cells which produce them and contain them. The fixatives, in this respect, approach sucrase (invertin): these various soluble ferments pass readily into the fluids which bathe the cells that produce them. The fixatives are found not only in the blood serums, prepared outside the body, but also in the blood plasma, whence they pass into the fluids of the exudations and transudations. Whilst no cytases are found in the subcutaneous tissue, or in the clear fluids of oedemas containing no, or almost no, cells, fixatives are not absent from these various situations just indicated. For this reason, when micro-organisms are introduced subcutaneously, they are not found to be altered by the cytases, but it is easily seen that they are permeated with fixatives. The same rule applies to the fixatives of the animal cells. In the example we have cited, the spermatozoa, in an animal whose serum renders these cells motionless, remain quite motile in the epididymis and below the skin. From this fact it may be concluded that these situations contain no free macrocytase. It is sufficient, however, to add to these motile spermatozoa a drop of normal serum containing macrocytase to stop their movements at once, the fixative being well distributed in the plasma of the living animal. The spermatozoa, then, were sensibilised by the fixative which was found in both the epididymis and in the subcutaneous tissue.

The cytases are soluble ferments which are essentially intracellular: the fixatives are, on the other hand, soluble ferments which are humoral. These fixatives, however, although circulating in the plasmas, are undoubtedly of cellular origin. This fact was first demonstrated by Pfeiffer and Marx, who found the specific fixative of cholera vibrios in the "haematopoietic organs," that is to

say, in the spleen, lymphatic glands, and bone marrow, at a period
[580] when there was, as yet, none in the blood. This fact has been extended to other examples of fixatives of micro-organisms, and it cannot be questioned that the phagocytes produce these soluble ferments. Under the influence of the introduction of micro-organisms into the body, a phagocytic reaction is produced which has, as a consequence, the digestion of these micro-organisms and the production of corresponding fixatives. There is every reason to believe that, in these cases, it is the microphages which, seizing and digesting the micro-organisms, produce the fixatives.

But the macrophages are also capable of producing these adjuvant ferments. Even in normal animals the macrophagic organs, such as the spleen, and especially the mesenteric glands, contain fixatives which help in the solution of the red blood corpuscles. Into this group of facts we must also place the production by the mesenteric glands, as well as by certain other lymphoid organs, and the leucocytes of exudations and the blood, of enterokynase,—the soluble ferment which aids the digestive action of trypsin. This enterokynase is also a species of fixative; it permeates the flakes of fibrin and renders them much more accessible to the influence of the trypsins.

The fact that the enterokynase of the intestinal digestion corresponds in so many respects to the fixatives which act in the absorption of formed elements in general and of micro-organisms in particular, furnishes a further proof that the destruction of micro-organisms in the animal is an act similar to true digestion.

Phagocytes, those elements which accomplish the absorption of micro-organisms and of animal cells, those holders of digestive cytases, are also the manufacturers of fixatives. Having brought about this absorption, the phagocytes set to work to elaborate large quantities of fixatives, although they are unable to increase the amount of cytases in any marked degree. The fixatives, produced in abundance, can be excreted outside the phagocytes and pass into the blood plasma, and, with it, into the fluids of exudations and transudations. But this excretion is not an indispensable act for the functioning of the fixatives. As these ferments prepare the way for the digestive action of the cytases, it is necessary only that they should be able to fix themselves on the formed elements before the latter. It is, therefore, easy to explain cases of acquired immunity in which no fixatives are found in the body fluids. Such examples

are not rare, and are characterised by the absence of any protective action on the part of the blood serum. In these cases, the fixatives, [581] whose existence is very probable, remain lodged within the phagocytes, just as are the cytases. Within these digestive cells the fixatives may quite well fulfil their preparatory rôle, this being followed immediately by the action of the cytase. The same rule may apply also to the cases of absorption in the unprepared animal, where fixatives are not found in the blood serum, but where they are able to act within phagocytes.

The excretion of fixatives into the plasmas, which constitutes the rule in cases of acquired immunity, presents an analogy with the excretion of pepsin into the blood. This soluble ferment can and does pass habitually from the stomach into the blood and thence into the urine, where it is often met with. As the pepsin, which only acts in an acid medium, cannot be utilised in the alkaline blood plasma, it is evident that its excretion is only the consequence of a too abundant over-production.

In recent years great attention has been paid to the essential mechanism of the action of fixatives on the formed elements on the one hand, and on the cytases on the other. According to Ehrlich, the fixatives are bodies intermediate between the two. In possession of two haptophore molecular groups, they are capable of entering into chemical combination with the micro-organisms or the animal cells on the one hand, and with the cytases on the other. It is for this reason that Ehrlich applies to them the name of "amboceptors" or "intermediary substances." Based on analogous examples in organic chemistry, Ehrlich thinks that the fixatives serve to introduce the cytases into the cells upon which they have to act. Bordet does not share this view and maintains that the action of the fixatives is not a chemical action in the proper sense of the word, but is a kind of mordanting which sensibilises the formed elements to the fermentative action of the cytases. According to him, the fixatives have no affinity for the cytases and in no way serve them as intermediaries, for which reason he gives to them the name of sensibilising substances. The question is still under discussion, but we may hope that it will soon enter into its final phase.

According to Ehrlich's theory, the fixatives contain no product coming from the micro-organisms or from the animal cells upon which they are fixed. The fixatives are, according to him, side-

chains or receptors, produced in excess and expelled into the blood
[582] plasma by the cells which produce them. Ehrlich does not tell us to what category these cells belong ; he maintains only that these cells must be in possession of receptors endowed with a specific affinity for certain molecular groups of micro-organisms and of animal cells. As soon as the receptors are saturated by these molecular groups, the cells which make use of the former for their nutrition produce them in superabundant quantity. The cells of animals, treated with micro-organisms and their soluble products, or with red blood corpuscles or any other kind of element of animal origin, acquire the property of elaborating more and more of the corresponding receptors, a large proportion of which are expelled into the blood plasma.

The common point between Ehrlich's theory and the view maintained in this work consists in the admission of a cellular property which develops more and more in proportion to the treatment of the animal by formed elements of all kinds. As, in acquired immunity against micro-organisms, the fixatives are most frequently found in the body fluids, it must be concluded that, in all these cases, the cells which produce them have become adapted by a kind of education to manufacture increasing quantities of fixatives. But even in those examples of acquired immunity where fixatives are not found in the plasmas, we must accept a modification of the cells which resist the invasion of micro-organisms. These changes in the cellular properties constitute, therefore, the most general, and consequently the most important, element in acquired immunity against micro-organisms.

As already mentioned Ehrlich does not assign any position to the cells which exhibit these modifications. It must, however, be accepted that they belong to the category of phagocytes. Indeed, the phagocytes put themselves into most intimate contact with the micro-organisms and foreign animal cells, and it is in the phagocytic organs that the fixatives are found before they are met with in the blood plasma. It may then be concluded that, in acquired immunity against micro-organisms, the phagocytes become adapted to elaborate the fixatives in large quantities, of which a portion is excreted into the body fluids, as has been shown in many examples of such immunity.

The progressive adaptation of the phagocytes in intracellular digestion can be demonstrated by the fact that in an immunised

animal the fixatives are found more especially in the phagocytic organs. The leucocytes which digest gelatine exhibit in an even [583] more distinct fashion the modification of these cells in animals which have received several injections of gelatine. The leucocytes of exudations, when the fluid is removed, become much more fitted to digest the gelatine than they were at first.

A similar adaptation is also observed in intestinal digestion, which may serve as a fresh point of comparison between the intracellular digestion of the phagocytes and the extracellular digestion in the intestines. The pancreas, in order to secrete its soluble ferments, adapts itself to the nature of the food which passes into the digestive canal.

The fixatives are not the only soluble ferments which appear in large quantities in the fluids of the immunised animal. Very often there are found along with them substances which agglutinate the micro-organisms in animals which have received several injections of micro-organisms of the same or an allied species. The same fact is observed in animals treated with animal cells. Thus the fluids of animals injected with blood corpuscles become agglutinative for these corpuscles.

The analogy between the agglutinins and the fixatives is so great that for some time several observers assumed them to be one and the same substance. This can no longer be upheld, for it is clearly demonstrated that the property of the body fluids to agglutinate micro-organisms and animal cells is different from that which brings about their permeation by fixatives. The agglutinins resist the same temperatures as the fixatives; both are specific to the same degree and pass equally from the cells which produce them into the plasmas of the blood, lymph, exudations, and transudations. The agglutinins capable of clumping the formed elements into masses may, under certain conditions, render their ingestion by the phagocytes more easy. In general, however, the part played by the agglutinins in acquired immunity must be regarded as of little importance, and for that reason we abstain from basing any theory of this immunity on the agglutinative property of the body fluids. Besides fixatives and agglutinins, the fluids of an animal which has acquired immunity very probably possess other properties which must have a greater or less function in acquired immunity. Thus, we are often struck by the stimulating action of these fluids on the normal animal into which they are introduced. This stimulation is [584] especially manifested against the phagocytic reaction.

As, in the majority of cases of acquired immunity, the blood serum contains fixatives in considerable proportion, and as these fixatives aid the action of the cytases in a remarkable fashion, we can readily understand that the introduction of such a blood serum into a normal animal, unprepared by any vaccination, may bring about a great resistance against the corresponding pathogenic micro-organisms. The fixatives, injected with the serum, fix themselves with avidity upon the micro-organisms. These organisms may become a more ready prey to the phagocytes and be destroyed very rapidly. In particular cases, where the injection of microbial cultures sets up a phagolysis, enough cytases are thrown out to affect the microbes already sensibilised by the fixative. This is followed by a refractory condition of the animal proportionate, in general, to the amount of fixative serum that is injected. This kind of acquired immunity, conferred by serums or certain other body fluids rich in fixative substances, has often received the name of passive immunity. This term is only justified in those rare cases where the introduced serum itself contains a sufficient amount of cytases to destroy all the micro-organisms. Most often it is the normal animal which has to furnish this bacteriolytic ferment. Now, as in phagolysis the quantity given off is too small, it is to the co-operation of the holders of cytases, that is to say, to the phagocytes, that the animal must have recourse. The phagocytes, being susceptible cells, their co-operation can only be counted upon in cases where they exhibit a sufficient activity. When these elements are weakened by narcotics or by any other cause, they become incapable of intervening with efficacy and the animal falls a victim to the pathogenic micro-organisms, in spite of the more than sufficient amount of fixatives that was introduced.

In natural or acquired immunity, it is the resistance of the animal against the micro-organisms which plays the principal part. The introduction of toxins ready prepared is only done under artificial conditions, as in laboratory experiments. Hence we see that, under natural conditions, it is against the penetration of the micro-organisms that the animal must be protected. So soon as these producers of poisons can no longer maintain themselves in the immunised animal their toxic secretions do not come into play. It is for this reason that animals vaccinated against pathogenic micro-organisms do not suffer from intoxication, although they are
[585] by no means insusceptible to the microbial poisons. It is a fact

of the highest importance from the point of view of immunity in general, that the resistance offered to micro-organisms in no way implies insusceptibility to their poisons. The view has frequently been expressed that, in acquired immunity at least, the animal must first acquire immunity against the microbial toxins, after which the micro-organisms, deprived of their principal weapon, descend to the rank of inoffensive saprophytes. Such cases may be found, but it is none the less true that immunity against micro-organisms may be acquired independently of that against the toxins, and that this constitutes the general rule.

Immunity is much more readily acquired against micro-organisms than against their toxins. Hence, antimicrobial vaccination was accomplished by science before that against their toxins. In the early researches on this subject antitoxic immunity appeared to be very difficult of attainment, and it was only after the discovery made by von Behring, who inaugurated a new path in microbiology, that better results were obtained. Von Behring not only succeeded in immunising animals against some of the principal microbial toxins, he demonstrated the existence of specific antitoxins in their body fluids.

This very unexpected conception of antitoxins at once took root in science, for it has been possible, thanks especially to the remarkable works of Ehrlich, to extend it to toxins of non-microbial origin. We are already acquainted with a certain number of antitoxins which, however, are not comparable in number to the other antibodies. Amongst these, the fixatives have many points of analogy with the antitoxins. Like them, they are resistant to heat: they exhibit also a fairly marked specificity, and, like the fixatives, they are distributed in the plasmas.

In the presence of so many points of similarity with the fixatives, one is tempted to attribute to the two categories of antibodies the same origin. The elaboration of antitoxins by the phagocytic elements, accumulated in the blood and disseminated in the organs, appears, in fact, to be very probable. Certain facts bearing on the absorption of various toxins by the leucocytes, as well as the distribution of antitoxins in the animal body, speak in favour of this view. On the other hand, the impossibility of attributing the elaboration of antitoxins to cells attacked by the corresponding toxins is quite in harmony with the same hypothesis. This hypothesis is especially supported by the numerous facts which prove the [586]

readiness with which the leucocytes react against all kinds of poisons, microbial or other toxins, as well as against organic and mineral poisons, such as the alkaloids and the arsenical combinations. However, in spite of so many data which speak in favour of the phagocytic origin of antitoxins, it has been impossible to support this view by rigorous facts easy of interpretation, such as those which science possesses in support of the phagocytic origin of fixatives.

The antitoxins have acquired a very great importance in the artificial cure of toxo-infective diseases, the aim in these cases being to paralyse the action of the toxins already produced by the micro-organisms and absorbed by the diseased animal. But their function is less in the protection against diseases where the object to be obtained is a reaction against the micro-organisms before these are able to inundate the animal with their toxic secretions. It is for this reason that the immunity against toxins must, in the study of immunity, occupy a less preponderant place than does the immunity against micro-organisms.

As the micro-organisms placed in the refractory animal ultimately undergo a digestion by chemical substances elaborated by the phagocytes, so also the toxins undergo a chemical modification due to the presence of substances in the production of which the living elements of the animal play a large part. The direct action of antitoxins on the toxins, so well demonstrated, especially by Ehrlich's investigations, does not, however, exclude the intervention of living cells, which, though sometimes not very manifest, is in other cases very marked.

The reaction of the living elements against the microbial toxins and their allies leads to the production, and even the over-production of antitoxins. According to Ehrlich, these elements are the receptors, or side-chains, which, to a certain extent, pre-exist in the cells which are capable of elaborating the antitoxins. On entering into combination with the toxin molecules, the side-chains, which are indispensable for the nutrition of the cells, are reproduced in very large numbers. After having saturated, so to speak, the productive elements of the antitoxin, the superfluous side-chains escape from the cell and pass into the plasmas of the body fluids. This theory may be brought into harmony with the other theory, which maintains that certain elements of the animal, capable of acting on the complex molecules of microbial toxins and their allies, produce special soluble
[587] ferments, which digest the toxins whose introduction frequently

excites the hypersecretion of the ferments. Here we have something similar to the hypersecretion, by the glands of the stomach, of pepsin, a part of which passes into the blood in order to escape with the urine.

According to Ehrlich's theory, the antitoxins are only capable of neutralising the injurious action of toxins when the former are found dissolved in the body fluids. The same receptors which fix the toxins in the plasmas and thus prevent them from reaching the susceptible elements, bring about an opposite result when they are found inside the cells. In this latter case, the receptors, owing to their great affinity for the toxins, attract them and allow them to pass into the cells, in this way aiding the dangerous function of the toxophore group.

This is an ingenious idea, conceived to bring into harmony a certain number of observed facts. In the present state of our knowledge it cannot be subjected to rigorous experimental test. Many well-established facts, however, are not in complete accord with this hypothesis. According to it the antitoxic immunity resides exclusively in the body fluids ; the living cells, instead of acquiring immunity, become more and more susceptible. Under these conditions it is difficult to conceive of an immunity against poisons of the simplest organisms ; nevertheless, this certainly exists. A plasmodium, which becomes adapted to all kinds of toxic substances, acquires an immunity against them, and this is due to changes taking place in the living elements ; it is not the result of modifications in the toxic fluids which bathe them. This biological adaptation is observed in the case of physical factors which may interfere with the life of these primitive organisms.

On the other hand, it must be accepted that the living cells of a complicated and higher organism may also acquire immunity against toxins. The first example of this kind was shown in relation to the red blood corpuscles of mammals vaccinated against the toxic serum of the eel. Whilst the body fluids of immunised rabbits become antitoxic, their red blood corpuscles, when completely freed from the serum, in certain cases resist the action of the eel's serum. It must be admitted that in this example we have an acquired immunity of the cells similar to that met with in lower organisms.

A second example of the immunity of the red corpuscles was observed by Ehrlich and Morgenroth in goats prepared by injections [588] of the blood of other individuals of the same species. In this case,

according to these writers, no co-operation by antitoxin is met with. The body fluids of the goats do not become capable of neutralising the toxin of the haemolytic serum, whilst the red corpuscles themselves acquire an immunity against this toxin, an immunity entirely cellular. Ehrlich attempted to penetrate into the essential mechanism of the resistance of the red blood corpuscles on the supposition that these corpuscles, instead of reproducing their receptors, as when there is production of antitoxin, get rid of them entirely. Deprived of receptors, they can no longer be affected by the haemolytic cytase which, as Ehrlich maintains, only penetrates into the red corpuscles owing to the affinity of the intermediate substance (fixative) for the receptor. This hypothesis of the mechanism of acquired cellular immunity scarcely accords with the hypothesis of the special function attributed to the receptors in the nutrition of the living elements.

Cellular immunity can be most easily demonstrated in relation to the red corpuscles of the blood, as these elements are very numerous and are capable of being isolated and freed from the fluid in which they are bathed. For this reason, science does not as yet possess sufficiently exact data on the immunity of other cells in higher animals. Many facts, however, indicate that such immunity does exist. There are, indeed, living elements which only acquire immunity with great difficulty and very slowly. Such are the nerve cells, elements which are specially susceptible. Von Behring has strongly insisted on the fact that in animals subjected to repeated injections of bacterial toxins, the nerve centres not only do not become accustomed to their injurious action, but even acquire a hypersusceptibility which is often very great. The observation is perfectly accurate, but it is none the less true that this period of exaggerated susceptibility is followed by another, during which the susceptibility becomes less marked and ends by giving place to a true adaptation. We are, therefore, compelled to accept the fact that even the nerve cells are no exception to the general rule, but are able to acquire a diminished susceptibility to a poison.

Several facts of another series confirm this conclusion. In the study of the action of the nervous system one frequently has occasion to observe instances of adaptation. I will cite as an example
[589] the adaptation of animals to spinal concussion studied by Lépine[1]. By percussing the lumbar region of rabbits and guinea-pigs we may induce in them an immediate paraplegia. This is transitory, and

[1] *Compt. rend. Soc. de biol.*, Paris, 1900, p. 385.

lasts at most for a few hours. The phenomenon may be reproduced several times in the same animal. "But," remarks Lépine, "when these experiments are continued for several days or several weeks, striking always at the same level, we soon observe that the resistance of the animals to the blows increases very rapidly, and that excitations which, in normal animals, produce paraplegias of several hours' duration, produce no effect upon those which have been under experiment for several days." We have in this example a real adaptation of the spinal region when subjected to concussion.

Similar facts are known to everyone as an experience of daily life. We can become habituated more or less easily to all kinds of violent sensations. Light and very intense noises which, at first, excite exaggerated reflex actions are ultimately perceived without setting up the least movement. Even in the psychical sphere habit dulls painful feelings, and it is very probable that a whole gamut of adaptation, starting from unicellular organisms which accustom themselves to live in an unsuitable medium, up to cultured human beings who habituate themselves to a disbelief in human justice, will be found to rest upon one and the same fundamental property of living matter.

Regarded from this point of view, immunity becomes a very general phenomenon, passing far beyond the resistance offered by the animal to infective diseases. After all is said and done, it invariably reduces itself to that cellular susceptibility [irritability] which governs so many of the vital phenomena in plants and in animals. It is this susceptibility which impels the branch towards the light and the root towards the ground, and which guides the spermatozoon towards the ovum. From the very commencement of embryonic life the cells derived from the segmentation of the egg exhibit a marked susceptibility. Wilhelm Roux[1] observed that the earliest cells of the frog embryo, if they are separated by artificial intervention, guided by their positive chemiotaxis again come together. In the formation of the tissues cellular susceptibility plays an important undoubted rôle. The prolongations of the nerve cells direct themselves towards the organs of sense or towards the muscular fibres, according to their [590] specific susceptibility[2]. The mother-cells of the capillary vessels are also guided by susceptibility, when they go towards a new-formed

[1] "Ueber die Selbstordnung der Furchungszellen," in *Berichte d. naturwiss. Vereins zu Innsbruck*, 1893, Bd. XXI.

[2] Herbst, *Biol. Centralbl.*, Erlangen, 1894, 1895, Bde. XIV, XV; Forssmann, Ziegler's *Beitr. z. path. Anat.*, Jena, 1898, Bd. XXIV, S. 56.

tissue, or when they approach one another and come together in order to form a vascular loop.

The phenomena of the organism which bear the sharpest impress of their physical and chemical nature, also come under the influence of cellular "sensations." Thus, in gastro-intestinal digestion, the secretion of the active juice is subordinated to the control of the nerve centres and even of the psychic centres. The sight of various kinds of food stimulates, unconsciously, by reflex action the activity of different digestive glands. In the same way the contraction of the contents of the cells of a plant subjected to plasmolysis, brings about the secretion of acid in order to augment the osmotic pressure.

Susceptibility, whose part is so great in the phenomena of immunity, taken as a whole, is a general property of living beings, regulated by a common law. Thus, in the chemiotaxis of the lowest unicellular organisms, as in the movements and the osmotic reaction of plants, there is manifested the same psycho-physical law of Weber-Fechner which regulates our own sensations.

All cells are able, by modifying their function under the direction of susceptibility, to adapt themselves to changes in the surrounding conditions. All living elements are able, therefore, to acquire a certain degree of immunity. But, amongst all the cells of the animal body, the elements which have retained most independence—the phagocytes—most easily and first acquire immunity to infective diseases. These are the cells which betake themselves to situations where micro-organisms and their poisons make their appearance, and which manifest a reaction against them. The phagocytes of the immune organism ingest and destroy micro-organisms and absorb toxins and other poisons. The final act of the reaction of the phagocytes is constituted by the chemical or chemico-physical processes concerned in the digestion of the micro-organisms, with the help of cytases, assisted by the fixatives; in the defence offered against poisons the phagocytes must also exert a chemical action.
[591] Before these phenomena come into play, however, the phagocytes manifest phenomena which are purely biological, such as the perception of chemiotactic and other sensations, the migration towards menaced situations, the ingestion of micro-organisms and the absorption of toxins, and finally the secretion of substances to be utilised in intracellular digestion.

The immunity in infective diseases presents itself, therefore, as a section of cellular physiology, and especially as a phenomenon con-

cerned in the absorption of micro-organisms. This absorption being carried out by an act of intracellular digestion, the study of immunity comes into the chapter on digestion regarded from the general point of view.

As in the struggle of the body of the animal against infective agents the phagocytes play the principal part, it happens that in certain diseases the micro-organisms in order to manifest their morbific effect must be protected from the attacks of these defensive cells. It is for this reason that the cholera vibrio, which is not very injurious when introduced below the skin of the human subject, becomes very formidable when it succeeds in gaining access to the digestive canal. Incapable of maintaining a struggle against the phagocytes, the vibrio is able to overcome in the stomach and in the intestines without difficulty the obstacles which it here meets with. It is for this reason that the channel of entrance of the micro-organisms at times plays such a prominent rôle in immunity against infective diseases.

The question is often asked whether a theoretical study of immunity is capable of rendering service in the search for means of conferring immunity on the animal. It must not be forgotten that theory and practice frequently march side by side, but that sometimes they advance without very much regard for each other. Thus the first preventive inoculations against snake-bite, small-pox, and pleuro-pneumonia, attempted by laymen were evidently made independently of any theoretical ideas of any kind, but were guided by the purest empiricism. On the other hand, the theoretical researches on the nature and origin of ferments led to the discovery of vaccinations by means of micro-organisms and microbic products which have rendered immense services to practical medicine.

The discovery of antitoxins, so rich in practical applications, was influenced by theoretical researches on the mechanism of immunity. Von Behring began his important series of investigations on this subject with the study of the immunity of rats against the anthrax bacillus. It did not suggest itself to anyone to suppose that this [592]
question could have the slightest immediate practical interest; nevertheless, starting from this investigation, von Behring, after giving up the theory of the bactericidal property of the body fluids as a cause of immunity, advanced, step by step, to the discovery of the antitoxic power of the serums. When a study of the properties of the blood of

animals treated with the red corpuscles of another species was commenced, no one would have suspected that these researches would end in the discovery of new methods for the recognition of human blood in medico-legal researches, or in the interests of hygiene for the determination of the source of a milk. The cellular theory of immunity is, as yet, of too recent date for us to claim the right to expect it to have amongst its assets methods for purely practical application. Nevertheless, it has already been found to be of service in the investigation of problems very closely affecting medical practice. Lord Lister, the greatest surgeon of the nineteenth century[1], asked himself how it was that wounds could heal "by first intention under circumstances before incomprehensible. Complete primary union was sometimes seen to take place in wounds treated with water-dressing, that is to say, a piece of wet lint covered with a layer of oiled silk to keep it moist. This, though cleanly when applied, was invariably putrid within twenty-four hours. The layer of blood between the cut surfaces was thus exposed at the outlet of the wound to a most potent septic focus. How was it prevented from putrefying as it would have done under such influence if, instead of being between divided living tissues, it had been between plates of glass or other indifferent material?" "How were the bacteria of putrefaction kept from propagating in the decomposable film? Metchnikoff's phagocytosis supplied the answer. The blood between the lips of the wound became rapidly peopled with phagocytes which kept guard against the putrefactive microbes and seized them as they endeavoured to enter. If phagocytosis was ever able to cope with septic microbes in so concentrated and intense a form, it could hardly fail to deal effectually with them in the very
[593] mitigated condition in which they are present in the air. We are thus strongly confirmed in our conclusion that the atmospheric dust may safely be disregarded in our operations; and Metchnikoff's researches, while they have illumined the whole pathology of infective diseases, have beautifully completed the theory of antiseptic treatment in surgery." (*Rep. Brit. Ass.*, p. 27.)

We may even attempt to increase phagocytosis in surgical operations, especially in those on the peritoneal cavity, by there setting up an artificial aseptic inflammation, by means of various substances,

[1] "The Relations of Clinical Medicine to Modern Scientific Development," a discourse delivered at Liverpool in September, 1896. *Rev. scient.*, Paris, 1896, 4e sér. t. VI, p. 481; [*Rep. Brit. Ass. Adv. Sci.*, London, 1896, p. 3; *Brit. Med. Journ.*, London, 1896, Vol. II, p. 733].

innocuous in themselves, which attract a large number of leucocytes. In laboratory practice this method is in daily use for the purpose of increasing the resistance of an animal against intraperitoneal injections of various micro-organisms, and Durham has suggested the extension of the same method to human medicine. Certain surgeons have already made attempts in this direction.

The application of the cellular theory of immunity to researches on new micro-organisms of infective diseases has already been crowned with success. Nocard and Roux have attempted to cultivate in the animal body the virus of the pleuropneumonia of cattle. They selected the rabbit, an animal naturally refractory against this infection. On the supposition that, in this immunity, the phagocytes must play an important part as destroyers of the presumed micro-organisms, the idea suggested itself to them to withhold the virus from their voracity. With this object they filled sacs of collodion or of reed pith with pleuropneumonia virus, and introduced these sacs into the peritoneal cavity of rabbits. Some time after this operation these investigators were able to demonstrate in the contents of the sacs impregnated by the blood fluid of rabbits, immune animals, the development of specific micro-organisms, the smallest discovered up to the present. By means of cultivations of this micro-organism, obtained in suitable media, they worked out a method of vaccinating animals which, as mentioned in Chapter XV., has already begun to give good results in veterinary practice. This method has thus contributed to the prevention of diseases, a branch of knowledge which has made such great advances since medicine became an exact science under the inspiration of the discoveries and ideas of Pasteur.

Within a very short period immunity has been placed in possession not only of a host of medical ideas of the highest importance, but also of effective means of combating a whole series of maladies of the most formidable nature in man and the domestic animals. Science is far [594] from having said its last word, but the advances already made are amply sufficient to dispel pessimism in so far as this has been suggested by the fear of diseases, and the feeling that we are powerless to struggle against them.

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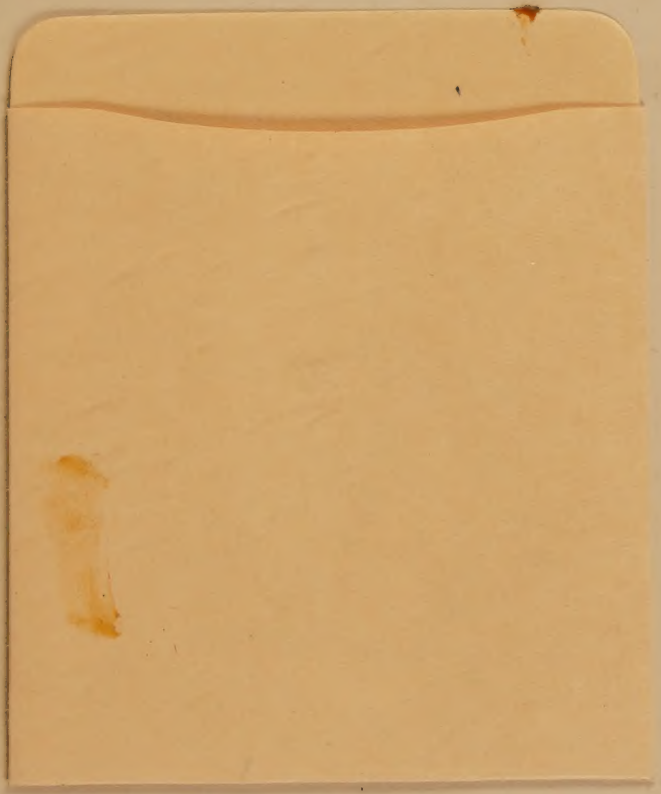
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